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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

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MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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ANNUAL MEETING, LOS ANGELES, CALIFORNIA, May 15–20, 1960

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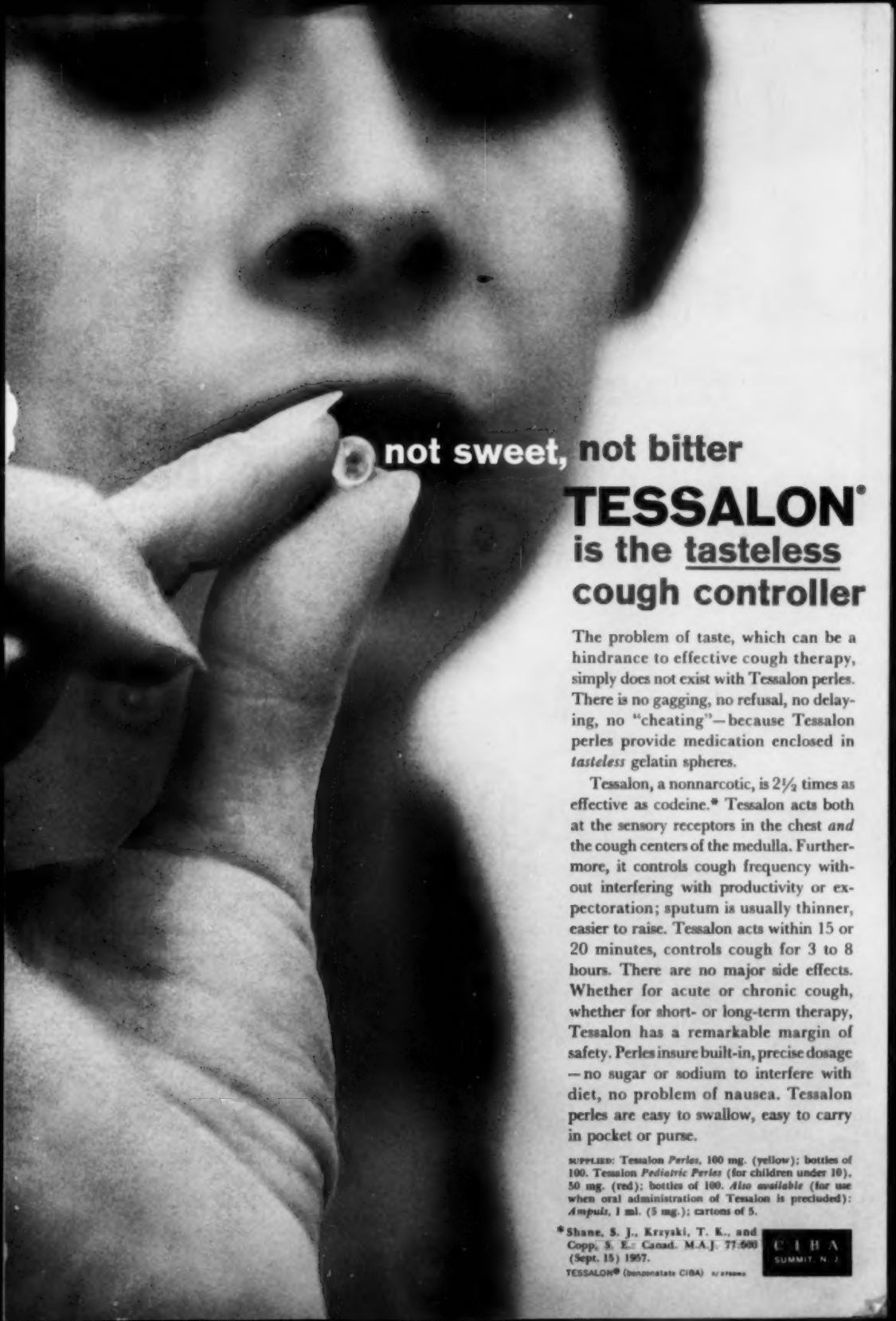

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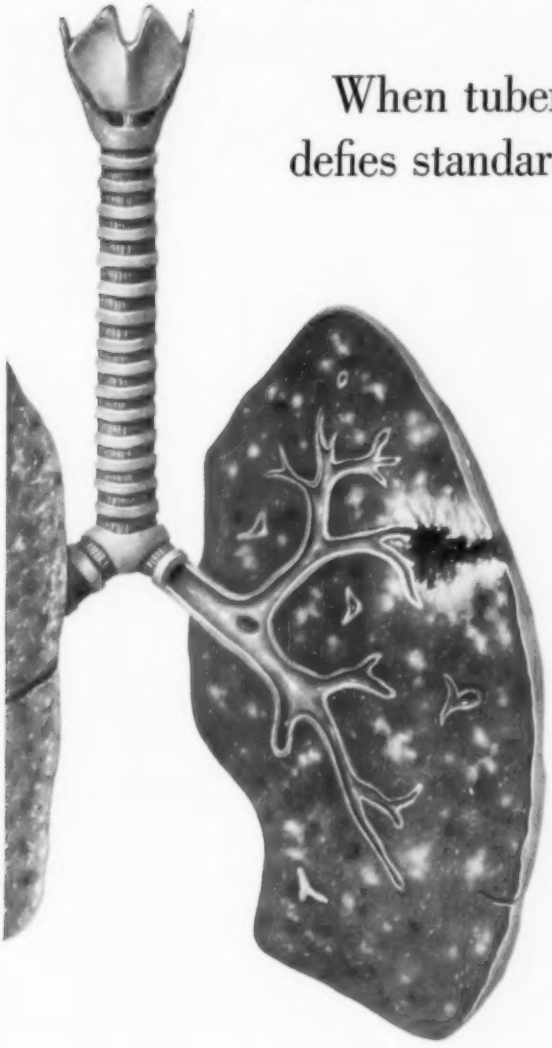

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1. Cohen, R. V., Lambert, R. L., and Molthan, L.: The Use of POTABA in Sarcoidosis; Annual Meeting American Trudeau Society, Eastern Section, Boston, Mass. (1958).

2. Zarafonetis, C. J. D., and Horrax, T. M.: Treatment of Peyronie's Disease with POTABA, Journal of Urology, 81: 770-772 (June 1959).

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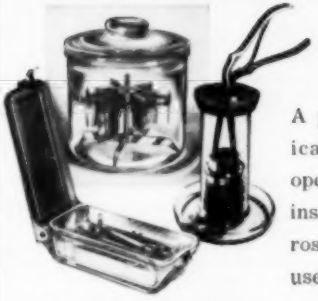

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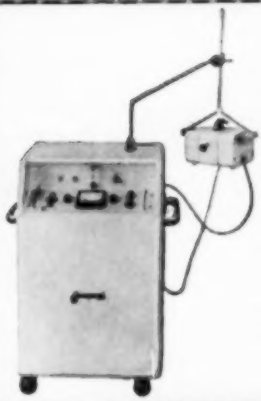

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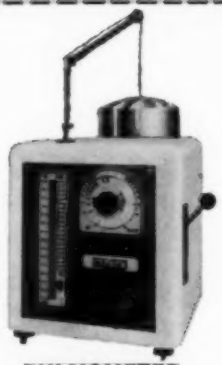

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PULMONARY VENTILATORY FUNCTION IN MILITARY RECRUITS DURING HEALTH AND ACUTE VIRAL RESPIRATORY DISEASE, INCLUDING PNEUMONIA[1, 2]

ROBERT B. STONEHILL,[3] NORBERT SCHALET, WILLIAM Y. FONG, HERBERT SALTZMAN, AND HAROLD B. HOUSER

(Received for publication June 25, 1959)

Introduction

Although acute respiratory disease among military recruits is of diverse etiology, many of the severe illnesses are accounted for by infection with adenoviruses types 4 and 7 (1). Pulmonary infiltration is present to some degree in approximately 20 per cent of recruits hospitalized with type 4 or 7 infections (2). The pathogenesis of the infiltrations is not clear. Because of the location and appearance of many of the lesions, some writers have attributed the pneumonias to aspiration of upper respiratory tract secretions (3, 4). The evanescent nature of the lesions and their appearance frequently give the impression that atelectasis precedes or is alone responsible for the roentgenographic appearance. Severe tracheobronchitis accompanies adenoviral infections in many instances, and it seemed possible that this might result in sufficient reduction in mechanical ventilation, because of pain, to result in atelectasis. Atelectasis combined with the bronchial secretions present might then result in the infection characterized clinically as atypical pneumonia.

To determine whether changes in pulmonary ventilatory function tests could be detected in the presence of acute respiratory disease, studies of ventilatory function were performed in Air Force recruits before, during, and after respiratory infections that included atypical pneumonia.

Methods

Sixty recruits who entered Sampson Air Force Base on the same day (December 1, 1955) and were formed into a single training flight comprised the study group. During the first five days after arrival, each man received, on the same day, a physical examination, a postero-anterior 6-foot roentgenogram of the chest, and pulmonary ventilatory studies. These procedures were repeated on each man during the second, fourth, seventh, and tenth weeks after the initial tests. In addition to the standard times for testing, the procedures were repeated in all men with respiratory disease at the time of illness. Daily sick-call was held and respiratory disease was considered present when any two of these findings were present: cough, sore throat, or oral temperature above 100°F. A diagnosis of pneumonia was made when definite peribronchial infiltration or confluent densities were present in the roentgenogram.

The pulmonary ventilatory tests were the vital capacity and the one-second and three-second vital capacities done on a Gaensler-Collins timed vitalometer, and a 12-second maximal breathing capacity done on a low resistance 9-liter Collins respirometer. The tests were considered satisfactory only when the subject had demonstrated maximal effort, which was defined as the repetition of the maximal breathing capacity to within 5 per cent and the vital capacity to within 3 per cent on any one test date. The formulas of Baldwin, Cournand, and Richards (5) were used to compare the predicted normal values for the tests.

At the beginning of the study each man received 600,000 units of benzathine penicillin G for prevention of streptococcal infections which were epidemic on the base.

[1] From the Pulmonary Laboratory and Medical Service, 3650th USAF Hospital, Sampson Air Force Base; and the Laboratory on Housing and Illness, Armed Forces Epidemiological Board; and the Department of Medicine, State University New York Upstate Medical Center; Syracuse, New York.

[2] This investigation was conducted under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and was supported by the Offices of the Surgeons General, Departments of the Army and Air Force, Washington, D. C.

[3] Present address: USAF Hospital, Lackland Air Force Base, Texas.

All men admitted to the hospital had cultures of the throat for β-hemolytic streptococci. Men with roentgenographic evidence of pneumonia had complement-fixation tests for psittacosis, Q fever, and adenoviral infection performed on paired serum specimens obtained at the beginning and completion of the study.

Results

The average age of the 60 recruits was 18.5 years (standard deviation 1.7 years), and the ages ranged from 17 to 25 years. The mean weight was 156.4 pounds (standard deviation, 18.4 pounds), and the range was 127 to 209 pounds. The mean height was 69.3 inches (standard deviation 2.2 inches), and the range was 64 to 74 inches. For the group of 60 men, the initially observed mean maximal breathing capacity of 140.3 liters per minute was 97.7 per cent (standard deviation 16.1 per cent) of the predicted volume; the mean vital capacity of 4.57 liters was 101.6 per cent (standard deviation, 10.2 per cent) of the predicted volume; and the mean one-second and three-second vital capacities were 87.2 per cent (standard deviation, 5.4 per cent) and 98.3 per cent (standard deviation, 1.8 per cent), respectively, of the vital capacities.

Although minor respiratory infections occurred in almost every man during the observation period, 26 men went through the period without having either respiratory infection meet the stated criteria or any roentgenographic evidence of pulmonary infiltration. Twenty men met the criteria for inclusion in the group with respiratory infections and had no evidence of pulmonary infiltration on roentgenography. Of the remaining 14 men, evidence of pneumonia was detected in 11 at the standard interval for roentgenographic examination, and in 3 on admission for respiratory infection. There was no significant difference among the three groups of 26, 20, and 14 men in any of the function tests performed at the initial testing except that the 20 men with respiratory infection had mean vital capacity values of 107.2 per cent (standard deviation, 10.2 per cent) of the predicted, compared with 98.9 per cent (standard deviation, 9.2 per cent) and 98.5 per cent (standard deviation, 8.9 per cent) in the other groups ($P = <0.01$).

The respiratory infections were typical of those described with adenoviral infection (2). Sore throat and cough were present in all men with respiratory disease. A productive cough was present in only one of the 19 men with respiratory disease without pneumonia, while the cough in the remainder was classified as nonproductive. This was in contrast to the presence of a productive cough in 7 of the 14 men with pneumonia. An oral temperature above 100°F. was present in 8 of the 19 men with respiratory disease without pneumonia, and in 3 of the 14 men with pneumonia. Three men whose pneumonias were discovered at the time of routine examination had no fever or sore throat, but one had a nonproductive cough and the other 2 had productive coughs. No substernal discomfort or wheezing was noted in any of the men at the time of testing of ventilatory function. Information about shortness of breath was not obtained.

Physical signs, except for marked injection of the pharynx, were scanty. In only 4 of the 14 men with pulmonary infiltrations were signs in the chest elicited, and these consisted only of rhonchi and subcrepitant inspiratory rales. Similar chest findings without roentgenographic changes were present in 5 of the 20 men with respiratory infections. The roentgenographic changes were confined to the lower lobes of the lungs and were usually near the cardiophrenic angles. Seven men had peribronchial infiltration and 7 had diffuse hazy densities. Two men who had pulmonary infiltration on their routine roentgenograms during the fourth week still had roentgenographic signs on the last day of observation, and 3 men had persistence of signs from the seventh week through the tenth week. In all other instances the roentgenographic evidence of pneumonia was present for no more than a week.

Eleven men had illnesses severe enough to require hospitalization. The presence of fever from 101° to 103°F. in these men was the only feature that distinguished them from the remainder of the men with respiratory symptoms. Only 3 of the 11 men had roentgenographic evidence of pneumonia, so that the presence of pulmonary infiltration did not appear to result in a more severe illness. No man was hospitalized for more than five days.

None of the men admitted to the hospital had group A streptococci in their throat cultures. Paired serum specimens from 10 men were available for antibody studies. There were no increases in complement-fixing antibody to psittacosis or Q-fever antigens. Three of 5 hospitalized men showed significant increase in adenovirus complement-fixing antibody, while none of 5 men not hospitalized showed an increase.

The serial observations of ventilatory function in the 26 men without respiratory disease are shown in table 1. The significance of changes in the mean values at the various periods of observation was tested by analysis of variance by subject and time. The only significant change ($P = <0.05$) was in the mean vital capacity at the four-week observation. The other mean values showed no significant variation for the five separate periods of testing.

Nineteen men with respiratory infection and normal roentgenograms had observations before, during, and after their illness. The mean values for these men are presented in table 2. Analyses of variance showed no significant difference in these values at any of the three periods of observation.

Ventilatory function before, during, and after evidence of pulmonary infiltration on roentgenographic examination was observed in 9 men. Roentgenograms of the remaining men with infiltrations had not cleared by the end of the observation period. The results of the tests in the 9 men are shown in table 3. The only significant change was in the mean vital capacity during illness, which showed a reduction of 0.49 liters ($P = <0.01$). Although the volumes of the one-second and three-second vital capacities also decreased during sickness, their percentages of the vital capacity values did not vary significantly from the percentages before and after

TABLE 1

MEAN MAXIMAL BREATHING CAPACITY AND VITAL CAPACITY BY TIME OF OBSERVATIONS DURING BASIC TRAINING FOR 26 USAF RECRUITS WITH NO OBSERVED DISEASE DURING TEN WEEKS OF OBSERVATION

Measurement	Time in Weeks after Basic Training Began				
	0	2	4	7	10
Maximal breathing capacity					
Total volume (liters per minute)	140.3	136.5	137.6	135.4	137.1
Per cent of predicted volume	99.8	96.2	96.6	95.2	97.0
Vital capacity					
Total volume (liters)	4.41	4.37	4.29	4.44	4.37
Per cent of predicted volume	98.9	97.2	95.2	98.0	97.4
1-second vital capacity					
Volume in 1 second (liters)	3.84	3.68	3.67	3.76	3.70
Per cent of total volume	87.5	85.0	85.5	85.3	85.3
3-second vital capacity					
Volume in 3 seconds (liters)	4.30	4.20	4.16	4.29	4.21
Per cent of total volume	98.7	98.1	98.0	97.9	96.8

TABLE 2

MEAN MAXIMAL BREATHING CAPACITY AND VITAL CAPACITY BY TIME RELATIVE TO DISEASE OF 19 RECRUITS IN BASIC TRAINING WITH RESPIRATORY DISEASE IN ABSENCE OF ROENTGENOGRAPHIC EVIDENCE OF PULMONARY INFILTRATION

Measurement	Before Sickness	During Sickness	After Sickness
Maximal breathing capacity			
Total volume (liters per minute)	144.1	147.3	148.2
Per cent of predicted volume	98.7	101.1	102.0
Vital capacity			
Total volume (liters)	4.86	4.88	4.85
Per cent of predicted volume	106.9	107.2	106.7
1-second vital capacity			
Volume in 1 second (liters)	4.11	4.03	4.05
Per cent of total volume	86.0	83.9	84.3
3-second vital capacity			
Volume in 3 seconds (liters)	4.72	4.72	4.71
Per cent of total volume	98.0	97.4	97.7

TABLE 3

MEAN MAXIMAL BREATHING CAPACITY AND VITAL CAPACITY BY TIME RELATIVE TO DISEASE OF 9 RECRUITS IN BASIC TRAINING WITH ROENTGENOGRAPHIC EVIDENCE OF RESPIRATORY DISEASE

Measurement	Before Sickness	During Sickness	After Sickness
Maximal breathing capacity			
Total volume (liters per minute)	132.7	125.6	133.1
Per cent of predicted volume	93.8	89.1	93.4
Vital capacity			
Total volume (liters)	4.50	4.01	4.38
Per cent of predicted volume	100.1	88.7	96.7
1-second vital capacity			
Volume in 1 second (liters)	3.81	3.29	3.57
Per cent of total volume	85.8	82.7	82.1
3-second vital capacity			
Volume in 3 seconds (liters)	4.31	3.74	4.13
Per cent of total volume	96.9	95.2	97.8

sickness. Five of the men had peribronchial infiltration and 4 had diffuse lesions. In all 9 men, the individual changes in vital capacity during illness ranged from no change in one man to a decrease of 1.35 liters in the man with maximal change. There was no positive correlation between degree of change and roentgenographic appearance.

DISCUSSION

The results on the first test date were of interest since all 60 recruits reported in normal respiratory health. The values of the maximal breathing capacity and vital capacity compared favorably with the predicted normal figures of Baldwin, Cournand, and Richards (5). The one- and three-second vitalometries conformed to the normal ranges described by Gaensler (6, 7). This concurrence suggested that the present population sample was not distorted by the selective action of Air Force enlistment. The initial testing of the two groups of men who subsequently developed respiratory disease or respiratory disease with pulmonary infiltration showed no variation from the group that remained free of disease except for vital capacity. The group that developed respiratory disease without pulmonary infiltration had a significantly higher initial mean vital capacity than the other two groups, and the mean value for this group remained higher throughout the observation period. Whether this was related in any way to their acquiring infections cannot be stated from these data. The 26 persistently healthy men displayed a stable ventilatory pattern on all five test dates with one exception: a statistically significant, but small, drop in the vital capacity occurred inexplicably during the fourth week of training.

The causes of the respiratory infections which occurred during the observation period were not satisfactorily elicited. The limited number of serum specimens available did not permit an estimate of the prevalence of adenoviral infection in the study group. However, demonstration of significant increases in antibody titer to adenovirus complement-fixing antigen in three of ten paired serum specimens indicated that adenoviral infections were present in the study group. Other agents undoubtedly were responsible for many of the infections, however. Psittacosis and Q fever were probably not responsible for any of the pulmonary infiltrations observed. Streptococcal infections were prevented by benzathine penicillin G. Although influenza antibody determinations were not done in these men, continued surveillance for influenza at Sampson Air Force Base during this period indicated the presence of influenza B infections on the base (8). It is likely that influenza B accounted for some of the infections. In general, the infections would be considered as moderately severe upper respiratory infection and mild to moderate atypical pneumonia. No bacterial pneumonias were recognized.

No significant changes occurred in the maximal breathing capacity or vital capacities during acute respiratory infections in the absence of roentgenographic changes. When such changes were present there was a significant reduction in the mean vital capacity. This change was of greater magnitude than that observed during the fourth week in the normal population. Even if

the reduction in vital capacity in the pneumonia group was the result of two things that were additive, such as the technical or mechanical factors responsible for change in the normal group and the same factors plus pneumonia in the pneumonia group, it still appears that the pneumonia and reduction in vital capacity are related.

There were no observations in the men with pneumonia during their respiratory infection before roentgenographic changes were detected. Thus the observations during illness were in men who did not develop pulmonary infiltration with their respiratory infection, or who had already developed infiltration at the time of testing. There was no opportunity to determine if the change in vital capacity preceded pulmonary infiltration or was only concomitant. Therefore, no information relating to the pathogenesis of the observed pneumonia in relation to ventilatory function tests was possible. It is difficult to explain the reduction in vital capacity on the basis of reduction in alveolar space as demonstrated by roentgenogram. The extent of involvement was minimal in most instances. Some other factor, such as bronchial secretions or restrictive chest movement, may have been at play.

The consistency of the serial observations in those recruits without respiratory disease indicates that there was a high degree of reproducibility in the ventilatory function tests. There were no changes that could be attributed to a learning process. Thus, one can have considerable confidence in doing serial observations on an individual and not expect significant variation in the results from technical aspects of the tests. This appears to be true even in the presence of mild to moderately severe upper respiratory infection in the absence of evidence of pulmonary infiltration. When the latter is present, there would appear to be no effect on the maximal breathing capacity, although the vital capacity may be reduced.

Summary

The pulmonary ventilatory function tests of maximal breathing capacity, vital capacity, and one-second and three-second vital capacities were performed serially at two- to three-week intervals for eleven weeks in a group of 60 Air Force recruits. Moderately severe acute respiratory disease occurred in 34 men, 14 of whom also had evidence of pulmonary infiltration of mild degree by roentgenographic examination. The remaining 26 men remained free of respiratory infection by the criteria established.

The only test that showed significant change at any of the periods of observation was the vital capacity. Although a significant decrease in vital capacity occurred on one occasion in the group without respiratory disease, those patients with pulmonary infiltration showed a more marked decrease during the time of their illness.

The reproducibility of the tests, even in the presence of respiratory disease without pneumonia, indicates that considerable confidence can be had that significant variation in the results will not occur in multiple tests in an individual because of technical reasons.

Sumario

La Función Ventiladora del Pulmón en Reclutas Militares durante la Salud y la Enfermedad Viral Aguda del Aparato Respiratorio, Incluso Neumonía

En un grupo de 60 reclutas de la Fuerza Aérea, se ejecutaron en series a plazos de dos a tres semanas durante once semanas pruebas de la función ventiladora del pulmón, comprendiendo la capacidad respiratoria máxima, la capacidad vital y las capacidades vitales de un segundo y de tres segundos. Había afección respiratoria aguda moderadamente grave en 34 sujetos, 14 de los cuales también revelaron en el examen radiográfico signos de leve infiltración pulmonar. Los 26 sujetos restantes permanecieron exentos de infección respiratoria, conforme a las pautas establecidas.

La única prueba que reveló mayor alteración en alguno de los períodos de observación fué la capacidad vital. Aunque hubo una disminución significativa de la misma en una ocasión en el grupo sin enfermedad del aparato respiratorio, los enfermos que tenían infiltración pulmonar mostraron una disminución más acentuada durante el período de su enfermedad.

La reproducibilidad de las pruebas, aun cuando existía afección respiratoria sin neumonía, indica que puede abrigarse mucha confianza de que no habrá, debido a razones técnicas, mayor variación en los resultados al ejecutar pruebas múltiples en un individuo.

Resume

Ventilation pulmonaire chez des soldats en bonne santé et des soldats ayant une infection pulmonaire à virus aiguë, y compris une pneumonie

On a effectué en série, à deux ou trois semaines d'intervalle, pendant onze semaines, chez des

soldats recrutés par l'Aviation, des examens portant sur la ventilation pulmonaire, la capacité maxima, la capacité vitale. Trente-quatre hommes eurent une infection respiratoire d'une gravité moyenne, 14 d'entre eux présentèrent aussi un petit infiltrat vu à la radiographie. Les 26 hommes restant ne présentèrent aucun symptome d'infection respiratoire d'après les critères fixés.

Le seul examen qui montra un changement important, à n'importe quel moment d'observation, fut la capacité vitale. Bien qu'une diminution notable de cette capacité se soit produit dans un cas du groupe sans aucune maladie pulmonaire, les malades avec un infiltrat montrèrent une diminution plus marquée pendant leur maladie.

Le fait que l'on peut reproduire ces examens même en présence d'une maladie respiratoire, sans pneumonie, indique qu'on peut être sur qu'il n'y aura pas, dans les résultats chez un même malade, des variations importantes pour des raisons d'ordre technique.

Acknowledgment

The statistical analysis of the present data was done by Dr. William F. Taylor, Department of Biometrics, School of Aviation Medicine, San Antonio, Texas.

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RESPIRATORY RESPONSES TO INTRAVENOUS SODIUM CYANIDE, A FUNCTION OF THE OXYGEN-CYANIDE RELATIONSHIP[1]

CONSTANTIN COPE[2] AND SOL ABRAMOWITZ

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Introduction

The fast intravenous injection of sodium cyanide in an adequate dosage in man almost invariably produces a deep inspiratory gasp. This fact was first investigated in detail by Loevenhart and associates (1) and was subsequently used for the determination of the circulation time by Robb and Weiss (2, 3). Heymans (4) discovered by cross-circulation experiments in animals that this respiratory stimulation of cyanide was mediated exclusively through the carotid and aortic chemoreceptors. At the intracellular level, the exact mechanism for this reaction is still a controversial one. It is known, however, that cyanide inhibits cytochrome oxidase in very low concentrations and, therefore, must depress aerobic intracellular respiration. Unmetabolized acid products consequently accumulate rapidly, stimulate the chemoreceptor nerve endings which send up volleys of impulses to the respiratory center, which is, in turn, reflexly stimulated to produce a gasp and hyperpnea.

Von Euler and associates (5) demonstrated in a very elegant manner the effect of sodium cyanide in anesthetized cats. He dissected afferent strands of nerve fibers from the carotid body and recorded directly their action potentials. A cyanide infusion was found to increase markedly the rate and amplitude of these impulses. With concomitant oxygen inhalation, however, it was noted that the traffic of impulses was greatly diminished or even abolished. It seemed, therefore, that pure oxygen is able to overcome in some way the tissue anoxia produced by a cyanide infusion (6).

A similar phenomenon was observed at the clinical level in this laboratory: oxygen breathing abolished the normal cyanide gasp in man. This paper will now attempt to elaborate further on this interesting topic.

Apparatus and Procedure

The apparatus used is a modification of the circuit described by Cournand and associates in 1940 (7). The patient breathes through a Collins five-way valve fitted with appropriate flutter valves. The source of oxygen or oxygen mixture is led to one arm of the apparatus via a 15-liter anesthetic bag so that inspiration is from the bag and expiration is to the outside air. Connected to the other arm of the five-way valve is a Collins 9-liter spirometer with soda-lime tower which is filled with the same gas mixture that is fed into the anesthesia bag. This system permits a patient to breathe an oxygen mixture from five to ten minutes, and then allows a switchover to the spirometer for recording at fast speed the reaction to the intravenous injection of sodium cyanide.

An 0.2 per cent sodium cyanide solution is prepared by dissolving 100 mg. of sodium cyanide (chemically pure) in 50 ml. of sterile saline on the same day as the procedure. This type of preparation was found to be sterile even after two weeks of incubation. The dosage given was fixed empirically at 0.1 mg. per kg., and was only increased if the gasp reaction was judged inadequate. The drug was administered rapidly within two seconds into the right antecubital vein with the arm at heart level and with the patient sitting up. The subject material consisted of patients judged to have no pulmonary disease by roentgenography and standard spirometry. The following combinations of tests were performed.

Observations

Patient breathing air: Within ten to seventeen seconds after the injection, most patients exhibit a sudden deep inspiratory response consisting of a gasp or a series of gasps with or without an elevation of the mid-chest position, lasting ten to twenty seconds; the minute ventilation may increase to 40 or 50 liters per minute in the

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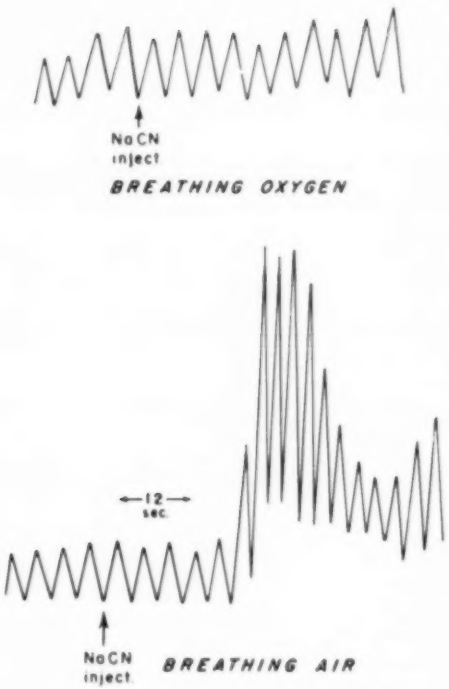


FIG. 1. Oxygen blocking of cyanide gasp.

first twenty seconds after the gasp, but then gradually returns to normal, usually within two minutes (figure 1). This reaction is completely involuntary and is usually accompanied by a subjective feeling of warmth in the chest and head. It is not unpleasant once the element of surprise is overcome. A long circulation time in normal patients is evidence of inadequate dosage (8). The dose is therefore increased in 1-mg. steps to a maximum of 10 mg. until a good response is obtained.

Patient breathing 100 per cent oxygen for ten minutes: Patients with no pulmonary disease have no gasp reaction whatsoever (figure 1). This will be described by the term "oxygen blocking." It is constant and reproducible. There seems, then, to be some competition between oxygen tension (9) and cyanide concentration. This could explain the phenomenon that doses of cyanide much lower than normal are required to produce a good gasp in patients with cardiac failure or pulmonary insufficiency (3); since these patients have a lower blood oxygen tension, it can be surmised that the traffic of impulses to the respiratory center from their chemoreceptors is heavier. It follows that a smaller amount of cyanide is necessary to increase the chemoreceptor stimulation to the threshold value where a gasp may be produced.

Inhalation of oxygen for five minutes after a cyanide gasp: Cyanide injection in this sequence usually does produce a gasp reaction, although often of a lesser magnitude than that produced with air. Full oxygen blocking is usually reestablished within ten to fifteen minutes, although in one subject it was not noted for more than an hour (figure 2). Loevenhart (1), by titrating an infusion of cyanide in human subjects, found the cyanide to be metabolized at the rate of about 1 mg. per thirty to forty seconds, or three to four minutes for an average dose. In this case, it is conjectured, therefore, that the intracellular metabolic derangement outlasts the disappearance of the cyanide.

Voluntary hyperpnea for twenty seconds before cyanide injection: In 2 patients, oxygen breathing did not block the gasp reaction. In order to exclude postural atelectasis and small mucous plugs in peripheral bronchi, the patients were instructed to cough and then to hyperventilate for twenty seconds. With this maneuver, oxygen blocking was successfully established (figure 3). The blocking could then be repeated fifteen minutes later without preliminary hyperventilation; this demonstrates that the hyperventilation alkalosis did not itself cause the blocking.

Effect of position on oxygen blocking: One markedly obese but otherwise normal patient was studied. In this case, oxygen blocking was normal in a sitting position, but could not be obtained in the supine position, even with preliminary hyperventilation. It is supposed, therefore, that the raising of the diaphragm by heavy abdominal contents can prevent adequate ventilation (10). This particular experience convinced us that all patients should be studied sitting up.

Effect of oxygen on respiratory stimulation produced by a cyanide intravenous drip: One hundred milligrams of sodium cyanide were dissolved in 250 ml. of saline, and the solution was given by intravenous drip at a rate sufficient to clearly increase the minute ventilation. Air and oxygen were given alternatively for five minutes at a time (figure 4); it can be noted each time

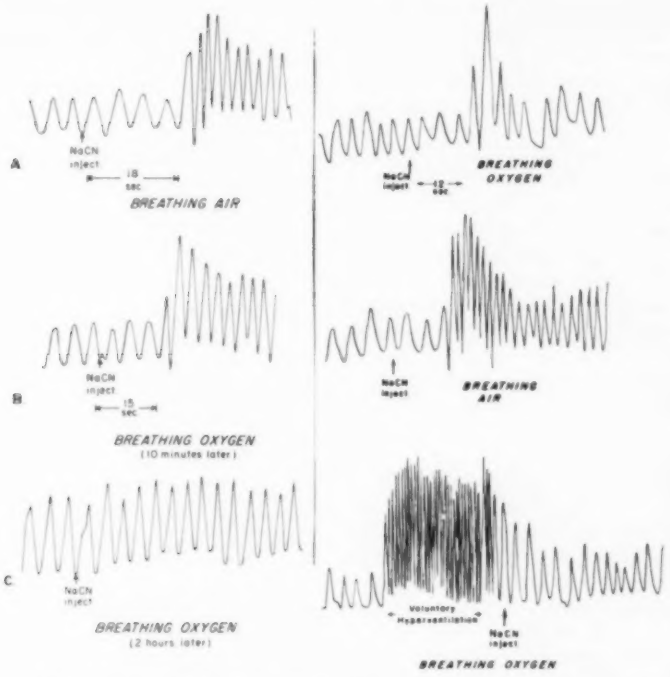
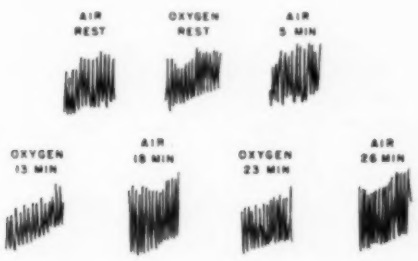


FIG. 2 (Left). Delay in oxygen blocking following a cyanide gasp.
FIG. 3 (Right). Oxygen blocking following hyperventilation.



	air	oxygen	air	oxygen	air	oxygen	air
TIME, min.	0	0	5	13	18	23	28
RESPIRATIONS / MIN	12	14	14	14	16	14	18
MINUTE VENTILATION, L	7.8	8.1	7.9	6.9	13.1	7.6	14.1
TIDAL VOLUME, cc.	646	577	568	490	820	548	783
OXYGEN UPTAKE / MIN, c.c.	208	216	234	244	263	230	300

FIG. 4. Continuous intravenous injection of NaCN: oxygen breathing brings minute ventilation back to control value.

that, within a minute, oxygen effectively blocks the cyanide respiratory stimulation.

Study of critical oxygen concentration required to produce blocking of the cyanide gasp: In this study, subjects were chosen who were less than forty years of age, were nonsmokers or light smokers, had no history of serious respiratory disease, no present upper respiratory infections, normal chest roentgenograms, normal vital capacity, timed vital capacity, and maximal breathing capacity. By studying a series of patients who met these standards on decreasing oxygen concentrations, it soon became apparent that oxygen in a concentration of about 30 per cent would block the cyanide gasp. It is difficult in practice to obtain commercially accurate oxygen-nitrogen mixtures. The 30 per cent oxygen gas tanks employed were actually found to contain 32 per cent oxygen.

In the present experience, it seems as if 30 per cent oxygen will block the cyanide response only in functionally normal lungs.

Twenty young patients with no evidence of pulmonary disease were found to be blocked by 30 per cent oxygen. There were 5 patients with histories of pleurisy, morning cough and expectoration, wheezing, and "smoker's" cough, but with otherwise normal spirographic studies and chest roentgenograms, who were blocked only by 40 per cent oxygen and not 30 per cent oxygen. However, 3 young patients were also found with minimally advanced arrested pulmonary tuberculosis who were blocked by 30 per cent oxygen.

In the cyanide study, there were 40 other patients with various pulmonary diseases such as emphysema, tuberculosis, sarcoidosis, and pleuritis. All had various degrees of pulmonary insufficiency. None were blocked by 30 per cent oxygen.

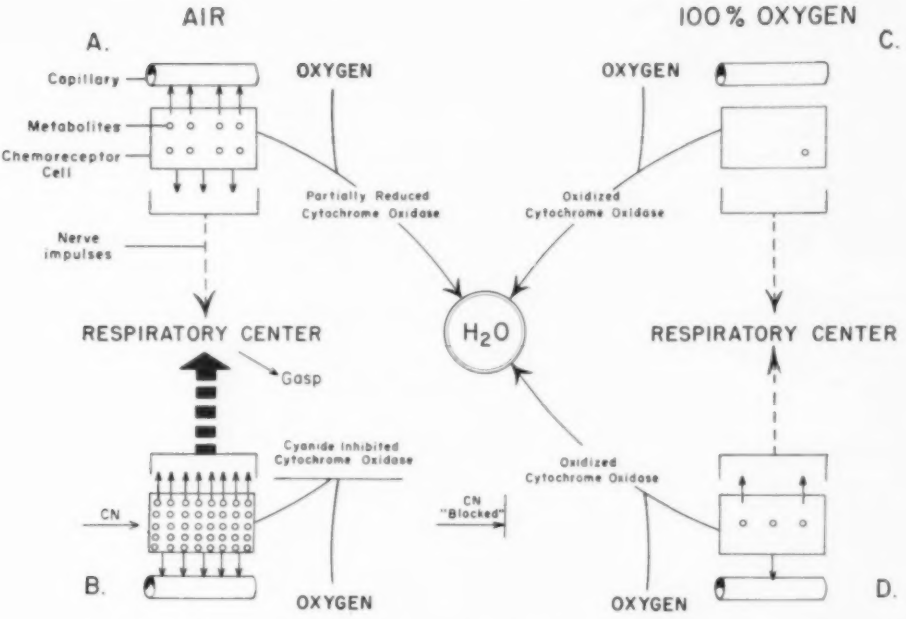
The experience is admittedly too small at this time to permit any definite conclusion from these data.

It is suggested, however, that the oxygen titration of the cyanide response shows promise as a pulmonary function test which may be sensitive enough for case finding in early emphysema, chronic bronchitis, pulmonary fibrosis, small vascular shunts, intracardiac right to left shunts, et cetera. Its use in screening flyers in whom normally functioning lungs may be critical at high altitudes may merit further investigation.

Discussion

All of the various tests described seem to show that there is competition for the chemoreceptors between oxygen and cyanide. A detailed investigation of the intracellular action of cyanide was first made by Warburg (11). He studied the fermentation of yeast cells suspended in sodium acid phosphate–acetic acid buffer, and showed that cyanide inhibition of cellular respiration could not be modified by altering the oxygen tension. It should be noted, however, that in these experiments he apparently did not attempt to vary the pH or temperature of the solutions or the concentration of the cyanide. There is also no mention of varying the duration of contact of cyanide with the yeast cells. On the basis of this work and, also, spectrographic evidence (12), it was concluded that cyanide combines with the oxidized form of cytochrome oxidase. This theory has been questioned by Wainio and Cooperstein in a recent review (13). They bring evidence to show that cyanide may combine with the reduced rather than with the oxidized form of cytochrome oxidase, and thus lay open once more the possibility of oxygen's competing with cyanide as it does with carbon monoxide. Thus, under low oxygen tension there would be plenty of cytochrome oxidase which could be inhibited by cyanide; contrariwise, under high oxygen tension most of the reduced cytochrome oxidase is changed by mass action to the oxidized form, which may in some way be protected from the cyanide. Obviously, much more biochemical work is required to test this hypothesis since it seems to be a very controversial subject. It is hoped, however, that the *in vivo* reactions described in this paper may help to shed some further light on this biochemical problem.

Stotz and associates (14) have shown that, at a normal intracellular oxygen tension of 40 mm., cytochrome oxidase activity may have only 60 per cent of its potential activity. Full activity is not reached until the intracellular oxygen tension is at least 70 mm. In other words, at least 30 mm. of extra oxygen tension may be required to saturate a cell from a respiratory standpoint. A diagrammatic representation of what may be happening in the carotid body cells is shown in figure 5. During air breathing, a certain backlog concentration of metabolites is present (15) which keeps stimulating the chemo-



POSSIBLE MECHANISM OF CN ACTION IN CHEMORECEPTOR CELL

FIG. 5. *A*. Backlog of metabolites stimulating a constant stream of nerve impulses to the respiratory center. *B*. Blockage of cytochrome oxidase by cyanide increases very rapidly concentration of intracellular metabolites; enough impulses are sent to respiratory center to exceed the threshold requirement for a gasp. *C*. Very few metabolites are present with 100 per cent oxygen. *D*. Cyanide given with oxygen seems unable to block the oxidized cytochrome oxidase to allow a sufficient concentration of intracellular metabolites to accumulate.

receptor nerve endings and causing a fine stream of impulses to be sent up to the respiratory center (figure 5*A*). During oxygen breathing, intracellular oxygen tension is well over 70 mm., oxidized cytochrome oxidase is brought into further activity, acid bodies are completely metabolized and, consequently, there is little to no intracellular backlog of metabolites to stimulate the intracellular nerve endings (figure 5*C*). If the cyanide is given at this time, even if some of the cytochrome oxidase is temporarily inactivated, there are not enough metabolites to reflexively stimulate the respiratory center to a gasp (figure 5*D*). It would seem, then, if this reasoning is correct, that raising the cellular oxygen tension about 30 mm. might be sufficient to block the cyanide reaction.

In order to do this in living beings, other barriers to the penetration of oxygen must be considered:

(*1*) The first barriers that come to mind are diffusion of oxygen through the capillary wall, the extracellular space, the cellular membrane, and the intracellular substance to reach the cytochrome system of the chemoreceptor cells. These factors are probably negligible.

(*2*) Local arteriovenous shunts as demonstrated by DeCastro (16), which may control the amount of blood perfusing the carotid chemoreceptor bodies. He showed *in vivo* that high oxygen tension and also adrenaline reflexively increase the flow through the chemoreceptor capillary meshwork, whereas breathing a high carbon dioxide concentration does the reverse. If these facts hold up in the intact being, then it cannot be said that a high oxygen tension reflexively shunts blood away from the glomus bodies and thus prevents cyanide from reaching the chemoreceptor cells in an effective concentration.

It can be supposed, however, that in those occasional instances when cyanide does not give a gasp reaction in air (3), the glomus bodies may be short-circuited for short periods of time. It is for this reason that, during the investigation of the

effect of various oxygen mixtures on the cyanide reaction, a control injection of cyanide while the patient is breathing air should always be performed.

(3) The lung. The factors affecting alveolar arterial gradient have been well worked out (17–20). With diffusion difficulty, the alveolar capillary gradient is usually negligible unless there is disease characterized by diffuse pulmonary fibrosis. Increasing the inspired oxygen tension by 70 mm. should overcome any generalized diffusing difficulty.

With the shunt factor, venous blood from the bronchial circulation and thebesian system dilutes the oxygenated blood by an estimated 2 per cent of the cardiac output. On this basis alone, the alveolar arterial oxygen pressure gradient may be 9 mm. when breathing air. When breathing 30 per cent oxygen, the gradient will also increase to about 20 mm. In certain diseases such as bronchiectasis and pleural thickening, the bronchial vessels may be very much enlarged; large bronchial anastomoses carrying only partially oxygenated blood empty into the left atrium and may greatly lower the arterial oxygen tension (21, 22). Similarly, congenital cardiac defects with right-to-left shunting will keep the arterial tension low even after breathing 100 per cent oxygen.

Uneven ventilation in relation to blood flow is probably negligible in perfectly normal ambulatory subjects, but becomes of great importance in common pulmonary diseases such as chronic bronchitis and emphysema.

It should also be remembered, however, that certain parenchymatous diseases of the lungs, such as tuberculosis, affect the vessels as well as the alveoli; the net effect is that there is no blood perfusing diseased lung and, thus, arterial oxygen tension may remain unaffected.

Hypoventilation resulting from decreased excursion of the breathing muscles may prevent the attainment of the critical opening pressure for many normal alveoli; as above, a condition is thus set up in which blood perfuses unaerated alveoli.

The total of the various factors affecting the oxygen tension gradient from the mouth to the intracellular respiratory enzymes of chemoreceptor cells when breathing air amounts to 10 to 20 mm. Moreover, as explained above, an additional 30 mm. of oxygen tension may be required to saturate fully the respiratory enzymes with oxygen; a more realistic value of 25 ± 15 mm. tension is assigned, therefore, since little is known about the factors which affect the metabolic rate in chemoreceptor cells. In other words, assuming normal arterial oxygen tension to be 100 mm., it can be said that the cyanide gasp will be blocked when the final blood oxygen tension is 125 plus-minus 15 mm. Inhalation of 25 to 30 per cent oxygen in nitrogen would meet these calculated requirements.

Since the present writers do not have any facilities at this time for accurately determining blood oxygen tensions, confirmation of these figures is not feasible.

If the present hypothesis proves to be correct, this procedure permits rapid and inexpensive evaluation, with different oxygen mixtures, of the ability of the lung to transmit oxygen and thus raise arterial blood oxygen tension to approximately 125 mm. In a way, it can be called an oxygen tolerance test in the same sense that a glucose tolerance test might measure the ability of the gut to absorb glucose.

Work is now in progress to evaluate various pulmonary diseases with this oxygen-cyanide test and to correlate these findings with standard spirometric tests.

Summary

Oxygen inhalation prevents the cyanide gasp in normal human lungs. There seems to be a competition between oxygen tension and cyanide concentration at the chemoreceptor sites. The critical oxygen concentration which will inhibit the cyanide gasp in functionally normal lungs is approximately 30 per cent.

Theoretic considerations are advanced to show that the cyanide-oxygen relationship may prove to be a useful index of the ability of the lung to transmit oxygen to the blood. This biologic phenomenon cannot, however, be used as a pulmonary function test until a much larger series of normal subjects and patients with pulmonary diseases are studied and the results are correlated with detailed blood gas analyses.

Sumario

Las Respuestas Respiratorias al Cianuro de Sodio por Vía Intravenosa: Función de la Relación Oxígeno-Cianuro

La inhalación de oxígeno impide el anhélito del cianuro en los pulmones humanos normales. Parece que hay una contienda entre la tensión de oxígeno y la concentración de cianuro en los sitios quimioreceptores. La concentración crítica de oxígeno que inhibirá el anhélito del cianuro en pulmones que funcionen normalmente es aproximadamente de 30 por ciento.

Se ofrecen consideraciones teóricas para demostrar que la relación de oxígeno-cianuro puede

constituir un índice útil de la capacidad del pulmón para transmitir oxígeno a la sangre. Sin embargo, no cabe usar este fenómeno biológico como prueba de la función pulmonar hasta que se estudie una serie mucho mayor de sujetos normales y de pacientes con afecciones pulmonares y se correlacionen los resultados con análisis pormenorizados de los gases sanguíneos.

Resume

Réponse respiratoire au cyanure de sodium intraveineux. Fonction de l'associçiation oxygène-cyanure

L'inhalation d'oxygène prévient normalement l'asphyxie par le cyanure dans le poumon humain. Il semble qu'il y a competition entre la tension d'oxygène et la concentration en cyanure au niveau des chimioreceptéurs. La concentration critique en oxygène qui inhibe l'asphyxie par le cyanure est normalement aux environs de 30%.

Des considérations théoriques ont tenté de prouver que l'assoçiation cyanure-oxygène peut être un index utile pour renseigner sur les possibilités de transport d'oxygène dans le sang au niveau du tissu pulmonaire. Ce phénomène biologique ne peut cependant pas être utilisé pour une étude de la fonction pulmonaire, tant que l'on n'a pas étudié des séries plus importantes de sujets normaux et de malades ayant des troubles pulmonaires, et, qu'on n'a pas comparé ces résultats avec des analyses détaillées des gaz du sang.

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BACTERIOLOGIC STUDIES OF THE SPUTUM IN PATIENTS WITH CHRONIC BRONCHITIS AND BRONCHIECTASIS[1, 2]

Results of Continuous Therapy with Tetracycline, Penicillin, or an Oleandomycin–Penicillin Mixture

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Introduction

Numerous reports of the flora in patients with chronic bronchitis and bronchiectasis have appeared from Holland (1–4) and England (5–11) in recent years. Only one study on a large number of specimens from patients with chronic bronchitis has been made in this country (12) and, so far as the writers could determine, none have been reported from a large number of patients with bronchiectasis. Since September, 1956, the present writers have been treating patients with chronic bronchial infections in succession with tetracycline, penicillin, a penicillin-oleandomycin mixture, or sucrose capsules as a control. A preliminary report of the clinical and bacteriologic results has been published (13). The group now numbers 89 patients; 63 of these were demonstrated by bronchogram to have bronchiectasis. Specimens of sputum from these patients were cultured at monthly intervals whenever the patient was producing sputum, and the results of these cultures are reported in the present paper.

Methods

Patients were included in this study if: (*1*) bronchography revealed one or more areas of bronchiectasis, and/or (*2*) they had had a history of chronic productive cough for one or more years and repeated lower respiratory tract infections without another reason for the cough and sputum.

Patients in whom no area of bronchiectasis could be demonstrated by bronchography were diagnosed as having chronic bronchitis. In the few cases in which bronchography could not be performed, the diagnosis of the respiratory illness was listed as undetermined.

As patients entered the study they were assigned in rotation to one of four treatment groups. Occasionally a patient was placed out of the strict order of rotation in order to make the groups more nearly uniform with respect to sex, age, and certain complications, such as hypogammaglobulinemia. All placements were made by one of the staff who had not examined the patients beforehand, and who did not make bacteriologic examinations of the sputum or tests of pulmonary function and did not participate in the evaluation of the clinical status of patients when they returned to the clinic.

One group of patients received 2 gm. of tetracycline daily in four divided doses one-half hour before each meal and at bedtime. During the first year of the study tetracycline hydrochloride was used while, in the second year, tetracycline hydrochloride with citrate buffer was substituted. A second group received 1 gm. of potassium penicillin G a day (1,600,000 units) in four divided doses. The third group received 2 gm. of a combination of oleandomycin and penicillin (1.3 gm. of oleandomycin–penicillin and 0.7 gm. of penicillin) a day in four divided doses. The fourth group received a sucrose placebo four times a day. All medications were dispensed in capsules of identical shape, size, and color.

Patients who discontinued therapy have been included in the tabulations up to the point when therapy was stopped. No patient has been excluded who remained on therapy for at least three months.

Sputum was collected in a sterile 60-ml. screw-capped bottle. A description of the specimen was made, recording the amount, color, consistency, et cetera. Early in the study, an unwashed pancreatin-digested sample (14) as well as a washed sample (3) were cultured. Later, the pancreatin digestion was discontinued and in its place an unwashed fleck of a portion of the sputum was used. The technique used was as follows: four sterile Petri dishes were set up, each containing 10 ml. of sterile physiologic saline. A generous portion of sputum was placed in the first dish and the fleck for the "unwashed" culture was taken

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TABLE 1

RESULTS OF SPUTUM CULTURES IN PATIENTS WITH BRONCHIECTASIS AND CHRONIC BRONCHITIS

Microorganism	Relation to Initiation of Therapy	Sputum Specimens Positive in Patients Treated with:							
		Placebo		Tetracycline		Oleandomycin-Penicillin		Penicillin	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total number of specimens	Before	42	100	49	100	46	100	45	100
	After	259	100	250	100	230	100	246	100
Hemophilus influenzae	Before	19	59	9	18	9	19	16	35
	After	109	42	34	13	70	30	116	47
Staphylococcus aureus	Before	9	21	4	8	6	13	6	13
	After	23	8	9	4	6	3	16	6
Diplococcus pneumoniae	Before	2	5	12	24	10	22	5	11
	After	48	18	5	2	4	2	11	4
Klebsiella-Aerobacter genera	Before	4	9	7	14	0	—	8	37
	After	25	13	48	19	33	10	52	21
Beta hemolytic streptococci	Before	0	—	1	2	2	4	1	2
	After	1	0.38	1	4	5	2	2	0.8
Proteus species	Before	3	7	2	4	5	11	5	11
	After	29	11	16	6	42	18	6	2
Pseudomonas species	Before	5	12	9	18	5	1.1	7	16
	After	16	6	44	18	12	5	16	7
Escherichia coli	Before	0	—	0	—	0	—	0	—
	After	9	3	7	3	8	3	10	4
Yeasts	Before	16	38	9	18	6	15	9	20
	After	71	27	61	24	35	15	65	26

Chi squares					
Before versus after therapy*:					
Staphylococcus aureus	χ^2 =	—	—	10.03	—
	p =	N.S.	N.S.	0.01	N.S.
Diplococcus pneumoniae	χ^2 =	—	35.7	31.84	—
	p =	N.S.	0.001	0.001	N.S.

		Placebo vs:		
		Tetracycline	Oleandomycin-Penicillin	Penicillin
After therapy*:				
Hemophilus influenzae	χ^2 =	51.09	5.39	—
	p =	0.001	0.05	N.S.
Staphylococcus aureus	χ^2 =	6.02	8.59	—
	p =	0.02	0.01	N.S.
Diplococcus pneumoniae	χ^2 =	37.27	43.50	22.75
	p =	0.001	0.001	0.001
Klebsiella pneumoniae	χ^2 =	—	—	5.14†
	p =	N.S.	N.S.	0.05
Proteus species	χ^2 =	—	4.90†	15.0
	p =	N.S.	0.05	0.001
Pseudomonas species	χ^2 =	26.5†	—	—
	p =	0.001	N.S.	N.S.

* Others not significant
† Unfavorable
N.S.—Not significant
χ^2—Chi square

from this dish. For the "washed" culture, a purulent fleck was taken from the "unwashed" dish and washed through three successive quantities of saline, then streaked on the plate. Another fleck was selected for each plate inoculated. The following media and methods of incubation were used: blood agar, penicillin blood agar, eosin-methylene blue, and Saboraud's agar incubated aerobically; blood agar and penicillin blood agar incubated under plus-minus 10 per cent carbon dioxide tension. In preliminary studies, parallel cultures were incubated aerobically and anaerobically; no strains were recognized in the anaerobic culture that were not also present in the aerobic culture. Further studies on anaerobic cultures will be carried out. A streak of penicillin-resistant staphylococci was made on all blood plates to aid in the growth and identification of *Hemophilus influenzae*.

After twenty-four hours' incubation, plates were examined for growth and relative colony counts were made. All organisms were identified by visual examination, subculture, and/or smear.

Staphylococci were tested for their ability to form coagulase by the tube test with rabbit plasma. All coagulase-positive strains were characterized by bacteriophage typing and production of alpha-hemolysin. Pneumococci were grouped (until serums were exhausted) and the microorganism injected into a mouse, which was killed and preserved in a frozen state for future study. Strains of *H. influenzae* were identified by indole production and growth on X- and V-factor media; they were typed by the quellung method with type B antiserum. An encapsulated type B strain was found in one specimen of sputum. Tube dilution susceptibility tests for penicillin, tetracycline, oleandomycin, and erythromycin were made on coagulase-positive staphylococci and all strains of *H. influenzae*.

Gram-negative rods were identified by differential media and biochemical tests. Yeasts were not differentiated but were reported as such. All other organisms were reported but not isolated, i. e., *Neisseria catarrhalis*, alpha and gamma streptococci, diphtheroids, et cetera.

Results

The frequency with which the various microorganisms were cultured from the specimens of sputum is shown in table 1. Bacteria that are considered to be common flora of the upper respiratory tract, such as alpha and gamma streptococci, *Neisseria catarrhalis*, and diphtheroids, have not been included. Since the trends were invariably the same in patients with bronchiectasis and in those with chronic bronchitis without bronchiectasis the two groups have been treated as one. The percentages of sputum specimens that contained *H. influenzae* decreased slightly during the period of therapy in the placebo and tetracycline groups, and increased slightly in the oleandomycin–penicillin and penicillin groups. None of these differences were statistically significant. The variation was probably due to the fact that only a few samples of sputum were obtained from a patient (sometimes only one) before therapy was started.

Comparison of the sputum specimens obtained after the beginning of therapy from patients who received placebo with those from patients treated with antimicrobial drugs shows interesting differences. Strains of *H. influenzae* were obtained from only 13 per cent of the specimens from the tetracycline group, and from 30 per cent of the specimens from the oleandomycin–penicillin group, as compared with 42

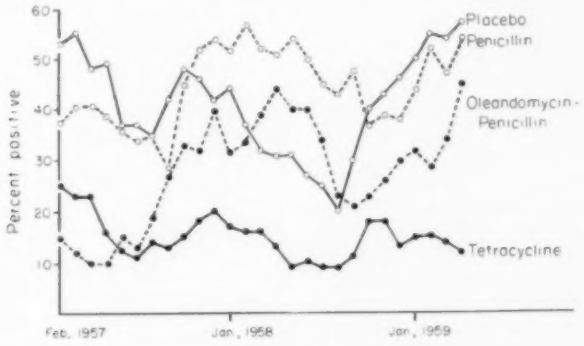

FIG. 1. Per cent of sputum specimens from patients with bronchiectasis and chronic bronchitis from which strains of *Hemophilus influenzae* were cultured (five-month moving average).

per cent from the placebo group. These differences were significant (P = <0.001 and <0.05, respectively). The frequency of isolations from the penicillin group was greater (47 per cent) than that from the placebo group, but not significantly so.

These differences are more striking if the seasonal variation is taken into consideration. From figure 1 it may be seen that *H. influenzae* was cultivated more frequently in the colder months of the year. Furthermore, the percentage of positive cultures from patients in the placebo and the penicillin groups remained higher in general than those in the other groups. *Hemophilus influenzae* was cultured infrequently from the patients treated with tetracycline at all times, although some seasonal variation was still evident. The percentages of positive cultures in the oleandomycin–penicillin group were between the penicillin and placebo groups, on the one hand, and the tetracycline group on the other.

It may also be seen in table 1 that the frequency with which *Staphylococcus aureus* was cultured from the sputum decreased after the beginning of therapy in all of the treatment groups, although the difference was not statistically significant except in the case of the oleandomycin–penicillin group (P = <0.01). If the groups are compared with each other after the start of therapy, both the tetracycline and the oleandomycin–penicillin groups showed significantly fewer staphylococci in the sputum than did the placebo group (P = <0.02 and <0.01, respectively).

The frequency of pneumococci decreased after therapy in all of the drug-treated groups and increased in the placebo group, but the differences were significant only for the tetracycline and oleandomycin–penicillin groups (P = <0.001). When the drug-treated groups after therapy were compared with the placebo group, the differences were significant for all three groups (P = <0.001).

TABLE 2

NUMBER OF PATIENTS WITH BRONCHIECTASIS AND CHRONIC BRONCHITIS FROM WHOSE SPUTUM CERTAIN MICROORGANISMS WERE CULTURED AT ANY TIME AFTER THERAPY WAS STARTED

	Relation to Therapy	Treatment Group: Placebo		Tetracycline		Oleandomycin–Penicillin		Penicillin	
		Number	Per Cent of Patients	Number	Per Cent of Patients	Number	Per Cent of Patients	Number	Per Cent of Patients
Total number of:									
Patients studied		23	100	21	100	21	100	24	100
Sputum cultures		301	—	289	—	276	—	291	—
Number of patients whose sputum contained at any time:									
H. influenzae	Before	10	44	8	38	7	33	12	50
	After	15	65	8	38	13	62	19	79
S. aureus	Before	9	39	4	19	5	24	4	17
	After	5	22	5	24	4	19	7	29
Pneumococcus	Before	2	9	7	33	6	29	4	17
	After	13	56	2	9	3	14	4	17
Beta hemolytic streptococci	Before	0	—	1	5	2	10	1	4
	After	1	4	1	5	3	14	1	4
Klebsiella–Aerobacter genera	Before	4	17	3	14	0	—	6	25
	After	11	48	12	57	10	48	13	54
Proteus species	Before	2	9	1	5	3	14	3	13
	After	3	13	3	14	9	43	3	13
Pseudomonas species	Before	5	22	4	19	3	14	6	25
	After	5	22	9	43	7	33	6	25
Yeasts	Before	11	48	7	33	4	19	7	29
	After	11	48	11	52	9	43	10	42

TABLE 3

RESISTANCE TO SEVERAL DRUGS BY STAPHYLOCOCCI ISOLATED FROM SPUTUM OF PATIENTS WITH BRONCHIECTASIS AND CHRONIC BRONCHITIS

Drug Tested	M.I.C.* More than 2γ per Ml.	Treated with: Placebo			Tetracycline			Oleandomycin–Penicillin			Penicillin		
		Before Therapy	After Therapy		Before Therapy	After Therapy		Before Therapy	After Therapy		Before Therapy	After Therapy	
			1 to 6 Months	More than 6 Months		1 to 6 Months	More than 6 Months		1 to 6 Months	More than 6 Months		1 to 6 Months	More than 6 Months
Tetracycline	Yes	3	2	2	1	4	4	6	2	2	2	5	5
	No	3	5	5	3	1	1	2	0	0	3	5	6
Penicillin	Yes	4	5	5	1	3	4	5	2	2	2	7	7
	No	2	2	2	3	2	1	3	0	0	3	3	4
Oleandomycin	Yes	0	0	0	0	0	1	0	0	0	1	1	1
	No	6	7	7	4	5	4	8	2	2	4	9	10
Erythromycin	Yes	0	0	0	0	1	3	0	0	0	0	1	1
	No	6	7	7	4	4	2	8	2	2	5	9	10

* M.I.C.—minimal inhibitory concentration.

None of the other microorganisms showed a significant increase or decrease following therapy. Strains of the Klebsiella-Aerobacter genera were significantly less frequent in sputum from the penicillin-treated group than in sputum from the placebo group ($P = <0.05$). Microorganisms were significantly more frequent in the sputum from the drug-treated patients than in sputum from the placebo patients in the case of Proteus in the oleandomycin-penicillin group and Pseudomonas in the tetracycline group ($P = <0.05$ and <0.001, respectively).

The relationship of the therapeutic regimen to the presence of microorganisms in the sputum can perhaps be visualized better by tabulating the number of patients from whose sputum the organism was cultured at any time before therapy and after the beginning of therapy. This has been done in table 2. *Hemophilus influenzae* was cultured from 44 per cent of the patients in the placebo group before therapy and in 65 per cent after therapy. When all of the specimens of sputum were included in the tabulation, whether obtained before or after the patient was started on placebo therapy, it was found that *H. influenzae* had been cultured from the sputum of 19 (82 per cent) of 23 patients at some time during the period of observation. When only the 14 patients in the placebo group who had bronchiectasis were considered, *H. influenzae* was found to have been cultured from the sputum of 13 (93 per cent) at some time before or after the initiation of therapy.

The percentage of patients with *H. influenzae* in their sputum in the oleandomycin–penicillin and penicillin groups rose, after treatment was initiated, to approximately the same extent as in the placebo group. On the other hand, the percentage of patients with *H. influenzae* in their sputum remained the same (38 per cent) before and after the beginning of treatment with tetracycline. The increase observed in the placebo group and, presumably, the increase in the oleandomycin–penicillin and penicillin groups can be explained by the fact that most patients entered the study in the late summer and autumn and, therefore, their pretreatment specimens were obtained at a time when the sputum was less likely to contain *H. influenzae*. The failure of the percentage to rise after the initiation of tetracycline therapy appears to be evidence of the therapeutic effect of tetracycline.

Little variation was observed in the numbers of patients whose sputum contained *Staphylococcus aureus* before and after therapy. These microorganisms were found in 19 to 29 per cent of patients during therapy.

The number of drug-treated patients whose sputum contained pneumococci at any time after therapy was started was low, and especially in the tetracycline group, in which pneumococci were found in the sputum of only 2 patients during therapy as compared with 7 patients

TABLE 4

BACTERIOPHAGE GROUPING OF *Staphylococcus Aureus* OBTAINED FROM SPUTUM OF PATIENTS WITH CHRONIC BRONCHITIS AND BRONCHIECTASIS

Treatment Group		Bacteriophage Group				Type 80/81	No Type Obtained	Total
		I	II	III/M/IV*	Mixed			
Placebo	Isolates	1	2	12	9	0	2	26
	Patients	1	1	6	5	0	2	15
Tetracycline	Isolates	3	0	7	5	3	1	19
	Patients	3	0	5	4	2	1	15
Oleandomycin-penicillin	Isolates	0	0	10	3	3	2	18
	Patients	0	0	8	2	2	1	13
Penicillin	Isolates	11	0	13	3	2	4	33
	Patients	6	0	8	3	2	4	23

* There were no isolates that were lysed only by Group IV phage (42 E).

before therapy. In contrast, 56 per cent of the patients in the placebo group had pneumococci in their sputum at some time during the period of therapy.

Streptococci of the beta hemolytic variety were found only rarely both before and after therapy was begun. This applied uniformly to all four treatment groups.

Microorganisms of the Klebsiella-Aerobacter genera were observed with approximately the same frequency in patients on each therapeutic regimen: in 48 to 57 per cent of the patients. Although this represented an increase above the frequency with which these microorganisms were cultured before therapy was begun, the increase can be explained on seasonal variation since it also occurred in the placebo group.

Proteus organisms were cultured only infrequently before therapy. No significant increase occurred during therapy except in the oleandomycin–penicillin group in which the number of patients whose sputum contained Proteus rose from 14 to 43 per cent. In the case of Pseudomonas, on the other hand, an increase took place after treatment in two groups: the tetracycline-treated and the oleandomycin–penicillin-treated patients.

Contrary to expectations, yeasts were cultured in approximately the same number of patients on each of the four treatment regimens (42 to 52 per cent of the patients).

In table 3 are listed the strains of *Staphylococcus aureus* isolated from the sputum of patients in the various treatment groups according to whether or not they were inhibited by 2 γ of tetracycline, oleandomycin, or erythromycin per ml., or by 2 units of penicillin per ml. Except in the case of tetracycline, there is no evidence that the administration of any of the drugs was followed by an increase in the number of staphylococci resistant to that drug. If the tetracycline-treated patients are compared with the placebo-treated patients, 8 of 10 isolates from the tetracycline group were not inhibited by 2 γ or less of tetracycline, while only 4 of 14 from the placebo group were resistant. This difference is statistically significant ($P = <0.05$). In addition, 4 of 10 isolates from the patients treated with tetracycline were resistant to erythromycin, while all of 14 isolates from the placebo group were susceptible to erythromycin. This difference is also significant ($P = <0.05$).

After the first month of therapy, there was very little change in the distribution of the drug susceptibilities of the staphylococci isolated from the sputum of different patients, regardless of whether they received specific therapy. Among the 6 strains not inhibited by 2 γ of erythromycin per ml., 3 were also resistant to oleandomycin. Among the 4 strains resistant to 2 γ of oleandomycin per ml., only one was susceptible to erythromycin.

A similar analysis of the drug susceptibilities of the *H. influenzae* isolates showed that most of the isolates were inhibited by 2 γ of tetracycline per ml. and 2 units of penicillin per ml., while only 40 per cent were inhibited by 2 γ of oleandomycin per ml. No significant differences were observed in the susceptibilities of isolates before and after therapy with any of the antimicrobial drugs.

That there was no significant relationship of

the bacteriophage groups to the type of therapy administered is shown in table 4. Although staphylococci of type 80/81, the type which has been responsible for many epidemics, were found in all of the groups except the placebo group the frequency of Group *III* strains, which are more often found in patients who have been in hospitals or have been under antimicrobial therapy, was not appreciably different in the various treatment groups.

Discussion

Various factors are said to be implicated in the production of chronic bronchitis, among which repeated infections, allergy, atmospheric pollution, and cigarette smoking are probably most important. The inception of bronchiectasis is thought to be related to heredity, bronchial obstruction, and a single severe infection or repeated infections. Whatever the factors involved in the inception of these two conditions, infection must play an important part at or soon after the beginning, and certainly is a major factor in perpetuating these conditions. Mulder (1) first emphasized the importance of *H. influenzae* in chronic bronchitis and bronchiectasis, and he and his associates (2–4) have added further information which strengthened this concept.

In table 5 are listed the results of studies by

TABLE 5

Frequency with which Certain Bacteria were Isolated by Various Investigators from the Sputum of Patients with Bronchiectasis and Chronic Bronchitis

Writers	Reference	Clinical Diagnosis	Total Number of Sputum Specimens	Per Cent of Specimens Containing:				
				*H. Influenzae**	Pneumococcus	*S. Aureus*	*Kl. Pneumoniae*	No Pathogens
Allison *et al.*	5	Bronchiectasis	100†	63	11	9	occ.	7
Barach *et al.*	17	Chronic bronchitis and bronchiectasis	30	7	10	30	27	3
Stuart-Harris *et al.*	6	Chronic bronchitis	172	15‡	51	12		n.s.
Stuart-Harris *et al.*	6	Bronchiectasis	45	22‡	58	16		n.s.
May	8	Chronic bronchitis	54	28	57	13	5	n.s.
Brown *et al.*	12	Chronic bronchitis	83	51	74	2		n.s.
Allibone *et al.*	9	Bronchiectasis (children)	32†	84	n.s.	n.s.	n.s.	n.s.
Elmes *et al.*	10	Chronic bronchitis	60	63	13	0	0	27
Edwards	11	Chronic bronchitis	320	49	39	5	11	n.s.
Present series		Chronic bronchitis and bronchiectasis	301	43	17	11	13	39
		Bronchiectasis	203	53	14	10	12	32
			Total Number of Patients	Per Cent of Patients Harboring:				
				H. Influenzae	Pneumococcus	*S. Aureus*	*Kl. Pneumoniae*	No Pathogens
Franklin and Garrod	7	Bronchiectasis (children)	36	97	31	n.s.	n.s.	n.s.
Brown *et al.*	12	Chronic bronchitis	20	70	90	10	n.s.	n.s.
Allibone *et al.*	9	Bronchiectasis (children)	32	100	n.s.	n.s.	n.s.	n.s.
Present series		Chronic bronchitis and bronchiectasis	23	82	65	48	57	8
Present series		Bronchiectasis	14	93	57	50	50	0

* Including *H. parainfluenzae*
† Bronchoscopic aspirations
‡ *H. influenzae* noted only when present in large numbers
occ.—occasional
n.s.—not stated

various investigators on patients with chronic bronchitis and bronchiectasis. Investigators who made a careful search for *H. influenzae* in the sputum or bronchoscopic aspirates found them in a high percentage of specimens. Stuart-Harris and his associates, in their early study reporting specimens as positive only when the microorganism was present in large numbers (6), found *H. influenzae* in 15 per cent of the sputum of patients with chronic bronchitis. Later, Stuart-Harris in association with Brown and other workers (12), including in their tabulations all specimens in which any *H. influenzae* organisms were seen, reported these microorganisms in the sputum of 51 per cent of patients with chronic bronchitis and, when sputum from the same patients was examined repeatedly over a period of months, in 70 per cent of patients. These patients were studied in Albany, New York. May and Oswald (15) stated that *H. influenzae* was isolated in their laboratory from 80 to 90 per cent of patients with chronic bronchitis with purulent sputum, provided that enough specimens from each patient were examined.

In the present study, *H. influenzae* was found in a slightly lower percentage of specimens of sputum than was found by most of the workers in England, although the 53 per cent observed in sputum from patients with bronchiectasis was not much lower than the 63 per cent reported by Allison and associates (5). Allibone's (9) high figures of 84 per cent positive sputum in children with bronchiectasis, and 100 per cent in children with *H. influenzae* in the sputum at some time, may be accounted for by the age group involved, since children apparently carry *H. influenzae* in the upper respiratory tract more frequently than adults (16).

Pneumococci were reported to be present in 10 to 74 per cent of sputum specimens according to various observers. It has long been established that pneumococci are cultured more frequently if they are sought by more than one method, and especially if all specimens are inoculated routinely into mice. Since in the present study the primary isolations were made on blood agar plates, it would be expected that the presence of pneumococci might be undetected in those specimens in which the number of organisms was low. It is significant that, when a number of specimens had been cultured, pneumococci were found one or more times in the sputum of 65 per cent of the patients.

Staphylococcus aureus was reported by others in 2 to 30 per cent of sputum samples, and was found in 11 per cent in the present study. Beta hemolytic streptococci were found only occasionally in these patients. This finding is consistent with the infrequency of streptococcal infections in adults in recent years.

Neisseria catarrhalis, diphtheroids, and streptococci of the viridans and indifferent varieties are found frequently in cultures from the upper air passages in normal persons and are, therefore, not reported in most series. Data on other microorganisms which are potentially pathogenic in the bronchi, either as primary or secondary invaders, are not available in most reports. The Klebsiella-Aerobacter genera were found in about half of the patients at some time during therapy (*see* table 2). Since these microorganisms were present with equal frequency in placebo-treated and drug-treated patients, it is unlikely that this was the result of the antimicrobial therapy. This assumption is further substantiated by the observation that in most patients these bacteria were cultured from only one or two specimens. They were present in three or more specimens from 3 patients in the placebo group, 5 patients in the tetracycline group, 3 patients in the oleandomycin-penicillin group, and 4 patients in the penicillin group.

The administration of oleandomycin and penicillin in combination may have been responsible for the high percentage of Proteus cultured from the sputum of patients treated with these drugs. In 5 of the 9 patients, Proteus was present in three or more specimens.

The percentage of patients from whom Pseudomonas was cultured rose after therapy was begun in the tetracycline and oleandomycin–penicillin groups and remained stationary in the other two groups. While this may represent an effect of the particular drug administered, the organisms were cultured more than twice from only 3 of the tetracycline-treated patients and from only one of the oleandomycin–penicillin-treated patients.

"Superinfection" with fungi is one of the potentially serious complications of antimicrobial therapy. For this reason it is especially interesting to note that the percentage of patients whose sputum contained yeasts during therapy with any of the drug regimens was not significantly different from the placebo group. It has been a surprise to find that the organisms that are frequently the cause of "superinfection," such as

staphylococci, yeasts, and gram-negative rods, did not appear to increase as a result of antimicrobial therapy, and were rarely found consistently in sequential cultures.

Even the staphylococci that did appear in the sputum during therapy were not resistant to the drugs any more frequently than were those obtained from pretreatment cultures, except in the case of tetracycline-treated patients. In this group, more isolates that were resistant to the particular drug employed were found after therapy than in the placebo-treated group. When the sputum specimens from individual patients were examined, it was evident that none of the patients in the tetracycline group retained staphylococci in the sputum consistently. In the writers' experience, almost all erythromycin-resistant staphylococci are also resistant to tetracycline and penicillin, and are usually acquired in the hospital. Since most of the strains isolated in the patients after treatment with tetracycline were resistant to all of these drugs it is likely that these strains were acquired from the environment. The fact that this was observed only in patients treated with tetracycline speaks in favor of the effectiveness of tetracycline, alone among the drugs used, in preventing the implantation of strains susceptible to it.

It is interesting that staphylococci of phage-type 80/81 were present in the sputum of 2 patients in each of the three drug-treated groups, and yet had apparently no effect on the infection in any case.

In a previous communication (13) it was reported that illnesses involving the lower respiratory tract were less frequent in patients who received tetracycline and also in patients who received the oleandomycin-penicillin mixture, although to a lesser extent. Lower respiratory illnesses were more frequent in patients from whom either *H. influenzae*, *S. aureus*, or pneumococcus was cultured at any time than in patients from whose sputum these microorganisms were not cultured. The present survey of the bacteriologic findings in these patients after an additional year of therapy supports the previous assumption that tetracycline therapy was the most effective of the regimens used. Apparently this occurred as a result of diminution in the numbers of the "pathogenic" bacteria (particularly *H. influenzae*, *S. aureus*, and pneumococcus) and did not necessarily require complete elimination of any one or all of these pathogens. It is gratifying to report that effective antimicrobial therapy did not cause any important increase in other potentially pathogenic bacteria in the flora of the sputum.

Summary

Eighty-nine patients with bronchiectasis or chronic bronchitis were treated for periods from three to thirty-one months with tetracycline, penicillin, an oleandomycin-penicillin mixture, or sucrose placebo tablets. Bacteriologic studies of the sputum were made at monthly intervals.

Hemophilus influenzae, *Staphylococcus aureus* and pneumococcus were considered to be the most important pathogens. Tetracycline or oleandomycin-penicillin therapy caused a significant diminution in the frequency with which these microorganisms were cultured from sputum. Tetracycline was particularly effective in decreasing the frequency with which *H. influenzae* was found in sputum. No significant increase in other potentially pathogenic bacteria was observed except for Proteus in the oleandomycin-penicillin group and Pseudomonas in the tetracycline group. In none of the patients did these microorganisms appear consistently in the sputum after therapy. There was no evidence that any of the therapeutic regimens produced any significant shift in resistance to any of the antimicrobials used in the study on the part of the staphylococci isolated from any of the treatment groups, except that tetracycline-resistant strains implanted more frequently in the sputum of patients treated with that antimicrobial than tetracycline-susceptible strains.

A definite seasonal incidence was seen in the frequency with which *H. influenzae* could be cultivated from specimens of sputum, isolates being more frequent in the colder months of the year.

Sumario

Estudios Bacteriológicos del Esputo en Enfermos de Bronquitis Crónica y Bronquiectasia. Resultados de la Terapéutica Continua con Tetraciclina, Penicilina o Mezcla de Oleandomicina-Penicilina

A 89 enfermos que tenían bronquiectasia o bronquitis crónica se les trató durante períodos que variaron de tres a treinta y un días con tetraciclina, penicilina, mezcla de oleandomicina-penicilina o tabletas de un placebo de sacarosa. A plazos mensuales se hicieron estudios bacteriológicos del esputo.

El *Hemophilus influenzae*, el *Staphylococcus aureus* y el neumococo fueron considerados como los bacilos patógenos más importantes. La terapéutica con tetraciclina u oleandomicina-penicilina motivó una notable disminución en la frecuencia con que se cultivaban estos microbios del esputo. La tetraciclina se mostró en particular eficaz para decrecer la frecuencia con que se encontraba el *H. influenzae* en el esputo. No se observó mayor aumento de otras bacterias potencialmente patógenas, aparte del Proteus en el grupo de la oleandomicina-penicilina y del Pseudomonas en el de la tetraciclina. En ninguno de los enfermos aparecieron dichos microbios constantemente en el esputo después de la terapéutica. No hubo signos de que ninguno de los regímenes terapéuticos produjera la menor desviación importante en la resistencia a ninguno de los antimicrobianos usados en el estudio de parte de los estafilococos aislados de los varios grupos tratados, aparte de que se implantaron más frecuentemente cepas tetraciclinorresistentes en el esputo de los enfermos tratados con este antimicrobiano que cepas tetraciclinosusceptibles.

Se notó una incidencia estacional bien definida en la frecuencia con que podía cultivarse el *H. influenzae* de ejemplares de esputo, siendo más numerosos los aislamientos en los meses más fríos del año.

Resume

Etude bactériologique de l'expectoration de malades ayant une bronchite chronique et des bronchiectasies. Résultats d'un traitement continu par la tétracycline, la pénicilline et un mélange oléandomycin-pénicilline.

Quatre vingt neuf malades ayant une bronchite chronique et des bronchiectasies ont été traités, pendant une période de 3 à 31 mois, avec de la tétracycline, de la pénicilline et un mélange oléandomycin-pénicilline, ou avec des comprimés de sucrose. Les auteurs ont fait tous les mois des études de l'expectoration.

Les germes pathogènes les plus importants que l'on trouva, furent l'*Hemophilus influenzae*, le *Staphylococcus aureus* et le pneumococcus. Le traitement par la tétracycline a diminué nettement la fréquence à laquelle on a pu cultiver ces microorganismes dans le sputum. La tétracycline agit d'une façon particulièrement efficace sur l'Hemophilus. Les auteurs n'ont pas constaté de développement exagéré d'autres germes pathogènes, excepté du Proteus dans le groupe traité avec de l'oléandomycin-pénicilline et des Pseudomonas dans le groupe traité avec de la tétracycline. Chez aucun malade ces germes ne réapparurent dans l'expectoration, après le traitement. En étudiant la résistance de staphylocoques isolés chez un quelconque de ces groupes, on n'a pu mettre en évidence aucun changement de résistance vis à vis des antibiotiques utilisés dans cette étude. On a seulement observé que les souches résistantes à la tétracycline poussaient plus fréquemment dans les crachats des malades traités avec cet antibiotique, que les souches sensibles à la tétracycline.

Il y a une nette influence saisonnière dans la fréquence à laquelle on a pu trouvé l'*Hemophilus influenzae* dans les expectorations. Ce germe se rencontre plus fréquemment pendant les mois froids de l'année.

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THE USE OF IMPROVED MICROBIOLOGIC AND HISTOCHEMICAL TECHNIQUES IN THE PATHOLOGIC STUDY OF NODULAR PULMONARY GRANULOMAS[1]

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Introduction

Nodular pulmonary granulomas may be defined as all those localized, usually solitary, and asymptomatic lesions that are not the result of neoplastic, metabolic, developmental, protozoan, or vascular abnormality, and which are of clinical importance principally because of their roentgenographic similarity to malignancies. The proportion of granulomatous nodules among all solitary lesions which are clinically indistinguishable from primary malignancies varies from 25 (1) to 87 per cent (2), but will average 30 to 40 per cent in most series. As continued improvements in diagnosis have been achieved, an increasingly larger number of granulomas of the lungs, previously considered tuberculous or nonspecific, have been shown to be the result of mycotic infections (3–16).

The morphologic changes resulting from mycotic infections have been appreciated for many years, but the identification of the fungi most commonly responsible for pulmonary granulomas, notably *Coccidioides immitis* and *Histoplasma capsulatum*, was restricted largely to those cases in which positive cultures could be obtained or in which viable organisms were demonstrable with routine hematoxylin and eosin stains. With the introduction of periodic acid-Schiff (PAS) (17) and Gridley (18) stains and their application to pulmonary granulomas, a surprising percentage of granulomas were found to contain fungi. A further substantial increase in positive mycotic identification followed the introduction of the Gomori methenamine-silver stain (2). An analysis of nodular pulmonary granulomas removed at surgery over a five and a half-year period from January, 1954, through June, 1959, employing these histochemical preparations together with routine histopathologic and bacteriologic studies, constitutes the basis of this report.

Methods and Materials

Exploratory thoracotomy is performed at The Baylor University Medical Center on all patients in whom nodular pulmonary densities have been demonstrated roentgenographically, when a specific diagnosis cannot be established on clinical grounds and when the diagnosis of malignancy cannot be excluded. Generally these patients are more than thirty-five years of age, with solitary nodules of uniform density without calcification. In occasional patients less than thirty-five years, the atypical nature of the nodule necessitates surgical exploration. No cavitary or diffuse processes were included and all cases of extensive fibrocaseous tuberculosis were excluded from the study. However, 6 patients in this group had known tuberculosis with densities thought to be filled-in cavities.

Routinely, the pathologist examined the specimen upon its removal and, if there was no indication of neoplasia on gross or frozen-section examination, material was obtained for smears and cultures of fungi and bacteria with special cultures for *Mycobacterium tuberculosis*. Multiple histologic sections of the lesion were made, and acid-fast, fungal, and routine hematoxylin and eosin stains were prepared from each block of tissue. The acid-fast and fungal preparations were screened by high dry magnification, and positive identification was accomplished under oil immersion magnification.

Prior to 1950 no stains were used that were selective for fungi. In 1950 the periodic acid-Schiff (PAS) stain (17) and in 1955 the Gridley stain (18) were employed for the histochemical demonstration of fungi, but from 1958 to the present the Gomori methenamine-silver stain (2) replaced the earlier preparations. For the present study all granulomas removed during a five and a half-year period from January, 1954, through June, 1959, were restudied, using the Gomori methenamine-silver stain, and a comparison was made of the recovery rate of fungi with the rates obtained by the PAS and Gridley stains.

All pulmonary nodules were included when macroscopic and routine microscopic examinations suggested an infectious etiology. Grossly, these granulomas were usually solitary, well-

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circumscribed nodules of small size and were located in all areas of all lobes. Microscopically, most of the lesions had necrotic, usually caseous centers and chronically inflamed peripheral fibrous shells. Multinucleated Langhans' giant cells and macrophages were present in varying numbers. Also included were those localized fibrotic lesions with no necrosis but with granulation tissue or macrophages.

The demonstration of mature or ruptured spherules with endospores or extruded compactly arranged endospores was required for definite identification of *Coccidioides immitis*. *Histoplasma capsulatum* was diagnosed only when round or

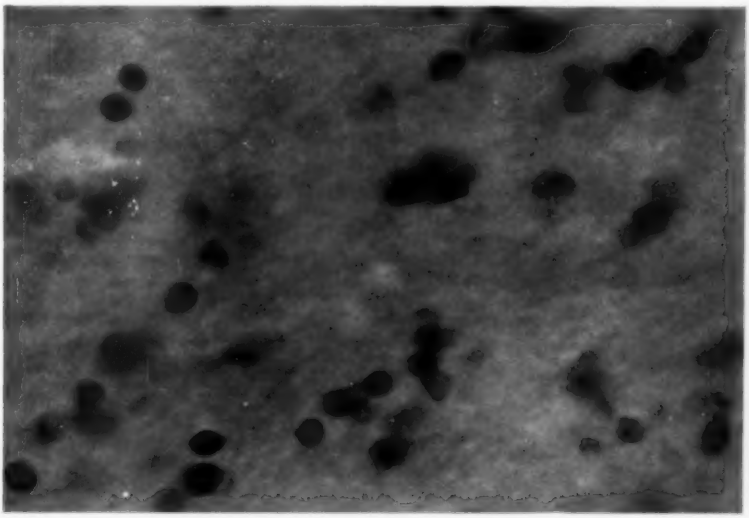

FIG. 1. *Histoplasma capsulatum* cells with peripheral capsule, central clear area and oval shape. (Gomori methenamine-silver stain, × 970)

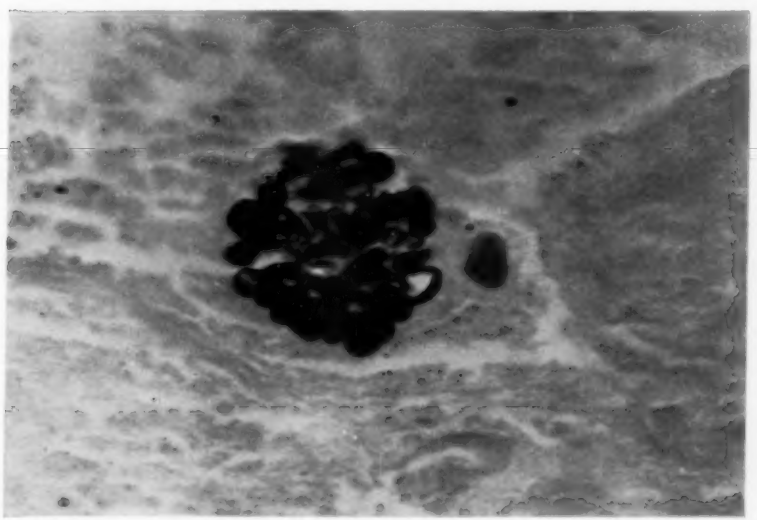

FIG. 2. *Coccidioides immitis* endospore cluster. (Gomori methenamine-silver stain, × 970)

oval yeastlike organisms, 1 to 5 μ in diameter reacted positively with the fungal stains. In the 2 cases of blastomycosis the cells averaged about 20 μ, were spherical or oval, and had distinct cell walls. Budding could be demonstrated in both cases. *B. dermatitidis* was cultured from one granuloma; no cultures were made in the second case. Typical "sulfur granules" were found in the tissues in actinomycosis. The single case of infection by *Nocardia asteroides* was diagnosed by the demonstration of nonacid-fast, Gomori methenamine-silver positive, branching bacillary organisms within a caseous granuloma.

When stained by the Gomori method, *Histo-*

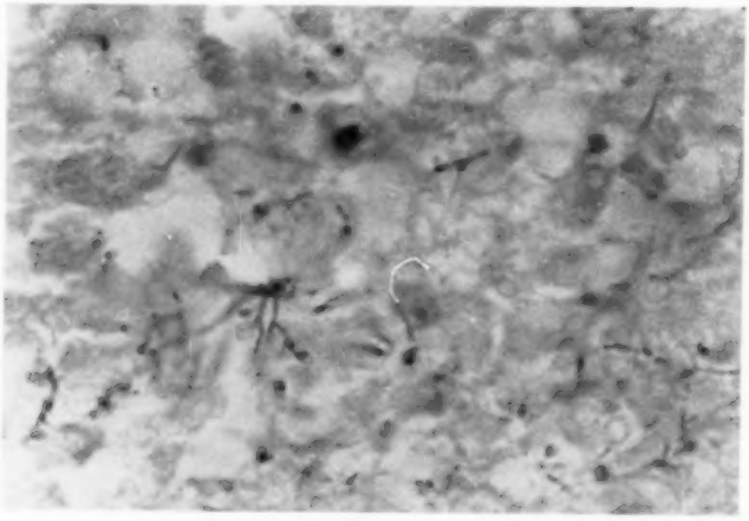

FIG. 3. *Nocardia asteroides* filaments. (Gomori methenamine-silver stain, × 970)

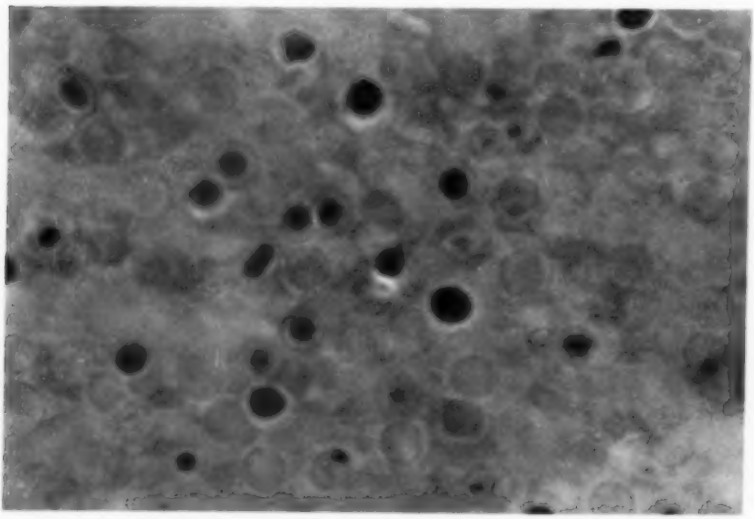

FIG. 4. Darkly staining homogeneous, rounded, erythrocytes. (Gomori methenamine-silver stain, × 970)

plasma capsulatum appears as a small oval cell with a grayish-black capsule and a clear central region (figure 1). Central dark bodies are rarely seen in contrast to the appearance of *Histoplasma capsulatum* stained by the Bauer, PAS, or Gridley techniques.

The present studies corroborated the observation of Straub and Schwarz (10) in that the organisms were typically found in the central area of the granuloma and were rarely identified at the periphery. They were present in loose groups and there was usually little variation in the size or shape of the cells; occasionally somewhat larger forms were seen. A definite differentiation was not always possible between the large forms of histoplasmosis and the smaller forms of blastomycosis and coccidioidomycosis. In the experience of the present writers, this did not constitute a major diagnostic problem.

Coccidioides immitis organisms were more typically found singly or in small compact clusters at the periphery of the necrotic central portion of the granulomas (figure 2). The circular capsule of the spherules and endospores stained brownish-black, and little central staining was seen. Endospore structure was well defined in the cells. No cases of cryptococcosis were encountered in this series. *Cryptococcosis neoformans* may resemble Coccidioides with the Gomori methenamine-silver stain but may be differentiated from it with the hematoxylin and eosin, mucicarmine, and PAS preparations in which the characteristic mucous capsules may be demonstrated. In the Nocardia granuloma the organisms were seen as branching elongated filaments and were identifiable only in the Gomori and Gram preparations (figure 3).

Substances other than fungi become impregnated with silver in most Gomori methenamine-silver preparations. Histiocytes containing jet-black anthracotic pigment are prominent. The pigment can be readily distinguished from fungi by its finely granular nature and irregular structure. Hemosiderin pigment stains brownish-black and is present in considerable quantities in the tissues of some lungs. Recognition of the intracellular nature of this material and its irregular distribution distinguish it from fungi. Erythrocytes occasionally will show a light brownish staining which may resemble histoplasmosis superficially (figure 4). However, the staining is relatively uniform in the central and peripheral regions of the cells, no sharply outlined capsule is seen, and erythrocytes are more rounded than *H. capsulatum*.

Observations

In the three-year period, 1946–1948, 23 granulomas of the lung were surgically excised and in only one was a fungus (Coccidioides) implicated (table 1). There were 6 positive identifications of *Mycobacterium tuberculosis*. Of 83 granulomas in the period 1954–1957, 18.1 per cent contained demonstrable acid-fast bacilli and 21.7 per cent were positive for fungi with the PAS and/or Gridley techniques. In the 1958–1959 period fungi were demonstrated in 63.1 per cent of 38 granulomas studied with the Gomori methenamine-silver stain. A comparison of the recovery rate of histoplasmosis during the latter two

TABLE 1

Original Pathologic Diagnosis of Nodular Pulmonary Granulomas

Period	1946–1948		1954–1957		1958–June, 1959	
Stains used	Acid-fast Bacilli		Acid-fast Bacilli, PAS, Gridley		Acid-fast Bacilli and Gomori Methenamine-Silver	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
Tuberculosis	6	26	15	18.1	6	15.8
Fungal disease	1	4	18	21.7	24	63.1
Histoplasmosis	0		0		14	36.8
Coccidioidomycosis	1	4	14	16.9	9	23.7
Blastomycosis	0		2	2.4	0	
Moniliasis	0		1	1.2	1	2.6
Actinomycosis	0		1	1.2	0	
Undiagnosed	16	70	50	60.2	8	21.1
Total granulomas	23	100	83	100	38	100

periods showed no cases from 1954 through 1957 and 36.8 per cent in the 1958–1959 period when Gomori methenamine-silver was employed as a routine stain in all granulomas. Diagnosed cases of coccidioidomycosis similarly rose from 16.9 to 23.7 per cent. There was a corresponding decrease in total undiagnosed granulomas from 60.2 per cent to 21.1 per cent.

These findings led to the re-examination with the Gomori methenamine-silver stain of 50 pulmonary granulomas in the 1954–1957 period which were reported as nonspecific after the routine hematoxylin and eosin, Gridley, and/or PAS and acid-fast stains. Whenever possible, sections from two paraffin blocks were selected from each case. The results showed a substantial number of fungi which stained clearly by this method (table 2). In each case the positive Gomori methenamine-silver-stained slides were compared with the original Gridley and PAS slides. In most cases no organisms were evident in the original slides, or very faint and equivocal traces of organisms were seen. Of the total of 50 previously undiagnosed granulomas, 42 per cent were reclassified as histoplasmosis; 22 per cent, as coccidioidomycosis; and 2 per cent, as nocardiosis. Thus, 66 per cent of the original group of granulomas classified as nonspecific were demonstrated to be specific. When these results were combined with the original positive diagnoses for the period 1954–1959, the rate of undiagnosed granulomas decreased to 20.6 per cent (table 3).

TABLE 2

RE-EXAMINATION OF UNDIAGNOSED GRANULOMAS, USING THE GOMORI METHENAMINE-SILVER STAIN, IN THE 1954–1957 PERIOD

	Number	Per Cent
Fungal disease	33	66
Histoplasmosis	21	42
Coccidioidomycosis	11	22
Nocardiosis	1	2
Undiagnosed	17	34
Total	50	100

TABLE 3

REVISED PATHOLOGIC DIAGNOSIS, USING THE GOMORI METHENAMINE-SILVER STAIN, IN NODULAR PULMONARY GRANULOMAS REMOVED FROM 1954–1959

	Number	Per Cent
Tuberculosis	21	17.4
Fungal disease	75	62
Histoplasmosis	35	29
Coccidioidomycosis	34	28.2
Blastomycosis	2	1.6
Moniliasis	2	1.6
Actinomycosis	1	0.8
Nocardiosis	1	0.8
Undiagnosed	25	20.6
Total granulomas	121	100

DISCUSSION

Until about fifteen years ago, an overwhelming majority of localized nodules of the lungs demonstrated roentgenographically, at surgery, or autopsy that contained necrotic or caseous centers were regarded as tuberculomas, despite the fact that a combination of preoperative cultures and bacteriologic and pathologic studies of the excised lesions revealed positive identification of *Myobacterium tuberculosis* in only about 20 to 25 per cent of the cases. The ensuing period has witnessed the development of three histochemical stains, the PAS (17), Gridley (18), and Gomori methenamine-silver stains (2). Their application to the histologic study of granulomatous pulmonary nodules has resulted in a progressive increase in the identification of etiologically significant fungi. Of these, the Gomori stain is currently the best histochemical preparation for the demonstration of fungi, as it is technically easy to perform, viable and nonviable forms of fungi can be demonstrated, distinct species of fungi can be accurately identified, and the results are more easily reproduced than they are with the PAS and Gridley stains. Thus, in the present study the addition of the PAS and/or Gridley stains to the histologic study reduced the nonspecificity of the pulmonary granulomas from 70 to 60 per cent, and the substitution of the Gomori methenamine-silver for the PAS and Gridley techniques was responsible for a reduction of nonspecificity to 21.1 per cent. Of 50 undiagnosed granulomas remaining after the use of the acid-fast bacilli, PAS, and/or Gridley stains, 66 per cent were demonstrated to be specific with the Gomori methenamine-silver staining.

To obtain the highest possible percentage specificity of pulmonary granulomas, it is important that a combined histopathologic and bacteriologic attack be made. As a routine, the pathologist should examine all pulmonary nodules

at the time they are excised. If gross examination and, when indicated, frozen-section examination have excluded the possibility of a neoplasm or developmental anomaly, an intensive search should be initiated to identify the causative organism. Material should be sent to the bacteriology laboratory for routine and acid-fast smears and culture for fungi and bacteria, including specific culture for *Mycobacterium tuberculosis*. Representative microscopic sections should be stained by the hematoxylin and eosin, the acid-fast, and the Gomori methenamine-silver techniques. A specificity in excess of 75 per cent should be obtained by this approach. The demonstration of one specific organism should not preclude attempts at identification of other agents; in one of the present cases, a combination of adenocarcinoma and histoplasmosis was demonstrated in separate nodules and, in two other instances, coccidioidomycosis and tuberculosis were diagnosed in the material from a single nodule.

Localization of tuberculomas was anticipated, chiefly in the upper lobes of the lungs. In contrast, the coccidioidal granulomas and histoplasmal granulomas showed no predilection for the upper lobes; in fact, all lobes were involved about equally. The apparent preference of coccidioidal granulomas for the right upper lobe and histoplasmal granulomas for the right lower lobe is not considered to be statistically significant. No conclusions can be drawn from the random distribution of the granulomas remaining unclassified. Unquestionably, new techniques will be developed which will result in the etiologic classification of these lesions (table 4).

In table 5 the relative proportion is shown of

TABLE 4

PERCENTAGE DISTRIBUTION OF NODULAR PULMONARY GRANULOMAS

	Right Upper Lobe	Left Upper Lobe	Right Middle Lobe	Right Lower Lobe	Left Lower Lobe
Tuberculosis (21 cases)	52.4	33.3	4.8	4.8	4.8
Histoplasmosis (35 cases)	20	20	2.8	37.2	20
Coccidioidomycosis (34 cases)	38.2	20.7	5.9	17.6	17.6
Undiagnosed (25 cases)	32	24	16	16	12

TABLE 5

PATHOLOGIC DIAGNOSES IN NONCALCIFIED SOLITARY PULMONARY GRANULOMAS REPORTED BY VARIOUS WRITERS

	Waldorf, Hutcheson		Segal, Starr, and Weed (13)		Schulz, Tucker, and McLoughlin (3)		Zimmerman (16)	
	Baylor Hospital, Dallas, Texas		Mayo Clinic, Rochester, Minnesota		Valley Forge Hospital, Rochester, New York		Armed Forces Institute of Pathology, Washington, D. C.	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Tuberculosis	21	17.4	3	3.2	18	40.8	6	17.2
Fungal disease	75	62	76	82.5	22	50.0	22	62.8
Histoplasmosis	35	29.0	67	73	16	36.3	19	54.2
Coccidioidomycosis	34	28.2	9	9.6	2	4.5	3	8.6
Cryptococcosis					1	2.3		
Blastomycosis	2	1.6			2	4.6		
Actinomycosis	1	0.8						
Nocardiosis	1	0.8						
Moniliasis	2	1.6						
Unclassified					1	2.3		
Brucella suis			1	1.1				
Undiagnosed	25	20.6	12	13.2	4	9.2	7	20
Total	121	100	92	100	44	100	35	100

classified and nonclassified solitary noncalcified granulomas in which a primary malignancy could not be excluded on clinical grounds, as reported by several writers. The relative incidence of tuberculosis, coccidioidomycosis, histoplasmosis, and the other fungal diseases will vary with the proximity of the hospital to areas where infections due to fungi are endemic and on the individual surgeon's criteria of operability.

Cultures were positive for *Mycobacterium tuberculosis* in 12 cases and negative in 4 cases among the 21 tuberculous granulomas. Cultures for fungi were attempted in 25 of the 35 histoplasmal granulomas and no organisms were grown. Of the 34 coccidioidal granulomas there were 9 positive and 11 negative cultures.

Summary

A combined histopathologic and bacteriologic approach in a large series of nodular pulmonary granulomas removed surgically because of possible malignancy resulted in the demonstration of specific infectious etiologic agents in a high percentage of cases.

Routine histopathologic studies, including acid-fast and Gomori methenamine-silver stains, together with routine bacteriologic studies for fungi and *M. tuberculosis*, were employed in this series. During a five and a half-year period from January, 1954, through June, 1959, a study of 121 granulomas revealed positive identification of *Mycobacterium tuberculosis* in 17.4 per cent; of *Histoplasma capsulatum* in 29 per cent; of *Coccidioides immitis* in 28.2 per cent; of *Blastomyces dermatitidis* in 1.6 per cent; of *Candida albicans* in 1.6 per cent; of *Actinomyces bovis* in 0.8 per cent; and of *Nocardia asteroides* in 0.8 per cent.

Sumario

El Uso de Técnicas Microbiológicas e Histoquímicas Perfeccionadas en el Estudio Histopatológico de los Granulomas Pulmonares Nodulares

Un aborde histopatológico y bacteriológico combinado en una numerosa serie de granulomas pulmonares nodulares, extirpados quirúrgicamente debido a la posibilidad de cáncer, dió por resultado el descubrimiento de específicos agentes etiológicos infecciosos en un porcentaje elevado de los casos.

En esta serie, se emplearon los estudios histopatológicos acostumbrados, comprendiendo las coloraciones ácidorresistente y la de metenamina-plata de Gomori, junto con los acostumbrados estudios bacteriológicos en busca de hongos y *M. tuberculosis*. Durante un período de cinco años y medio, desde enero de 1954 hasta junio de 1959 inclusive, el estudio de 121 granulomas reveló la identificación positiva del *Mycobacterium tuberculosis* en 17.4 por ciento; del *Histoplasma capsulatum* en 29 por ciento; del *Coccidioides immitis* en 28.2 por ciento; del *Blastomyces dermatitidis* en 1.6 por ciento; de la *Candida albicans* en 1.6 por ciento; del *Actinomyces bovis* en 0.8 por ciento; y de la *Nocardia asteroides* en 0.8 por ciento.

Resume

Utilisation de techniques histochimiques et microbiologiques améliorées dans l'étude histologique de granulomes nodulaires du poumon

Une étude histologique et bactériologique d'une importante série de granulomes nodulaires du poumon, ayant été réséqués chirurgicalement à cause de leur éventuelle malignité, a démontré l'existence d'une etiologie infectieuse dans un grand pourcentage des cas. On utilisa des études histopathologiques systématiques, comprenant des colorations pour bacilles acido-résistants et la méthode à l'argent-méthamine Gomori, en même temps que des études bactériologiques usuelles pour les mycoses et pour *M. Tuberculosis*. Pendant une période de 5 ans et demi, de janvier 1954 à Juin 1959, une étude de 121 granulomes a identifié d'une façon positive le *Mycobacterium tuberculosis* dans 17,4% des cas; Histoplasma capsulatum, dans 29%; *Coccidioides immitis* dans 28,2%; *Blastomyces dermatitidis* dans 1,6%; *Candida albicans* dans 1,6%; *Actinomyces bovis* dans 0,8% et *Nocardia asteroides* dans 0,8%.

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IX. PYRAZINAMIDE AND ISONIAZID USED IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1,2]

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Introduction

The use of pyrazinamide in the treatment of pulmonary tuberculosis has been the subject of extensive studies by the Veterans Administration-Armed Forces investigators of the chemotherapy of tuberculosis since the drug was first reported as a tuberculostatic agent in January, 1952, by Yeager, Munroe, and Dessau (1). Since 1954, these studies have been concerned chiefly with the evaluation of pyrazinamide used with isoniazid. A comparison of regimens containing pyrazinamide with other drug combinations has been made on the basis of randomized studies, using patients whose active pulmonary tuberculosis had not previously been subjected to drug therapy. By 1956 it appeared that perhaps the combination of pyrazinamide with isoniazid was a more effective therapeutic regimen than any of the double-drug regimens using isoniazid, para-aminosalicylic acid (PAS), or streptomycin. Further studies were therefore undertaken in an attempt to determine whether the combination of isoniazid with pyrazinamide would demonstrate sufficient therapeutic superiority to justify its use despite the hazard of hepatoxicity, which had been shown to result from the use of pyrazinamide. These studies were undertaken only in hospitalized patients who were subject to close supervision and a regular routine of liver function testing. This report presents the conclusions from certain of these studies.

Materials

Patients selected for the treatment-study program all had active cavitary pulmonary tuberculosis, had received no previous antituberculous drugs, and were producing tubercle bacilli, as demonstrated by sputum or gastric cultures. They were assigned by random allocation to one of the following regimens:

1. Isoniazid, 300 mg. daily, and para-aminosalicylic acid, 12.0 gm. daily; or
2. Isoniazid, 300 mg. daily and pyrazinamide, 3.0 gm. daily.

Previous reports have been made of preliminary observations (2) and from the first report of this study (3), but it is now possible to give more complete analysis of the results of the study.

Results

There are now 237 patients who were treated with isoniazid-PAS and 219 patients treated with isoniazid-pyrazinamide upon whom reports during the first four months of treatment are available.

Analysis of patients assigned to these regimens was made and it was determined that the groups were essentially comparable as to age, race, sex, extent of lung involved with disease, number and size of cavities, trend of disease, and presence of pre-existing collapse and surgical therapy.

Observations

Comparison of the effectiveness of the two treatment programs was made by recording the incidence of roentgenographic improvement (table 1), of cavity closure (table 2), and of reversal of infectiousness (table 3). This information is reported at four-month intervals. No consistent superiority of either regimen is evident on the basis of the roentgenographic change. Cavity closure was observed to occur somewhat earlier in the group treated with isoniazid-PAS, but only in the subgroup of patients with moderately advanced disease reported at two to four months is a difference of any significance noted. Although reversal of infectiousness had occurred slightly more rapidly with isoniazid-PAS, longer-term observation shows an insignificantly higher percentage of patients who received isoniazid-pyrazinamide with sputum negative for tubercle bacilli at the nine- to twelve-month period of observation.

[1] From the Veterans Administration Hospital, Oteen, North Carolina.

[2] Presented in part at the Seventeenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, February, 1958.

TABLE 1

ROENTGENOGRAPHIC IMPROVEMENT

	2–4 Months				5–8 Months				9–12 Months			
	Isoniazid–PAS		Isoniazid–Pyrazinamide		Isoniazid–PAS		Isoniazid–Pyrazinamide		Isoniazid–PAS		Isoniazid–Pyrazinamide	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total cases observed	230	100	210	100	153	100	109	100	71	100	43	100
Marked or moderate improvement	150	65	144	69	123	80	90	83	65	92	36	84
Slight improvement	66	29	59	28	20	13	15	14	1	1	7	16
No change	13	6	4	2	9	6	1	1	5	7	0	0
Deterioration	1	*	3	1	1	1	3	2	0	0	0	0

* Less than 0.5 per cent.

Comparison is made in each instance between pretreatment film and progress film taken during the stated interval.

TABLE 2

CAVITY CLOSURE

	2–4 Months				5–8 Months			
	Isonazid–PAS		Isoniazid–Pyrazinamide		Isoniazid–PAS		Isoniazid–Pyrazinamide	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total cases observed	237	100	219	100	159	100	115	100
Cavity closed	45	19	29	13	70	44	43	37
Moderately advanced cases observed	97	100	91	100	62	100	47	100
Cavity closed	34	35	19	21	37	60	25	53
Far advanced cases observed	140	100	128	100	97	100	68	100
Cavity closed	11	8	10	8	33	34	18	26

TABLE 3

BACTERIOLOGY: REVERSAL OF INFECTIOUSNESS

	2–4 Months				5–8 Months				9–12 Months			
	Isoniazid–PAS		Isoniazid–Pyrazinamide		Isoniazid–PAS		Isoniazid–Pyrazinamide		Isoniazid–PAS		Isoniazid–Pyrazinamide	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total cases	237	100	219	100	159	100	115	100	84	100	48	100
Conversion achieved	107	45	90	41	116	73	90	78	78	93	46	96
Conversion not achieved	130	55	129	59	43	27	25	22	6	7	2	4

An attempt was made to evaluate the relative effectiveness of PAS and pyrazinamide in preventing the emergence of bacilli which were resistant to isoniazid (table 4). Here again the early results favor isoniazid-PAS, and the late results are slightly better with isoniazid-pyrazinamide. The small number of patients in both study groups who are producing bacilli which show any growth in the presence of isoniazid remains a noteworthy finding from this aspect of the study. It is apparent that both PAS and pyrazinamide when given with isoniazid largely

TABLE 4

RESISTANCE TO ISONIAZID

	0–4 Months				5–8 Months				9–12 Months			
	Isoniazid–PAS		Isoniazid–Pyrazinamide		Isoniazid–PAS		Isoniazid–Pyrazinamide		Isoniazid–PAS		Isoniazid–Pyrazinamide	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total cases	195	100	184	100	150	100	110	100	78	100	46	100
Culture negative for tubercle bacilli	102	53	107	58	134	89	102	92	73	93	45	98
Culture positive for tubercle bacilli	93	47	77	42	16	11	8	8	5	7	1	2
Growth on control tube only*	89	45	64	35	12	8	2	2	2	3	1	2
Growth, isoniazid susceptible	2	1	5	3	1	1	2	2	0	0	0	0
Growth, isoniazid resistant	2	1	8	4	3	2	4	4	3	4	0	0

* No growth on any isoniazid-containing tube.

Isoniazid susceptibility defined: Less than equal growth in 1 γ per ml. tube and no growth in 5 γ per ml. tube.

Isoniazid resistance defined: Equal growth in 1 γ per ml. tube or less than equal growth in 1 γ per ml. tube with any growth in 5 γ per ml. tube.

prevent the emergence of isoniazid-resistant organisms.

Toxicity: Toxicity sufficient to require discontinuing PAS occurred in 19 patients, or 7 per cent of 267 who originally started to take isoniazid and PAS. This developed principally during the first four months of treatment and consisted of gastrointestinal intolerance in 13 cases and hypersensitivity reactions in 6 cases.

Pyrazinamide therapy was discontinued in 35 patients, or 13 per cent of 261 who were given isoniazid-pyrazinamide, because of toxicity attributed to the pyrazinamide. In addition, therapy was discontinued in 2 patients because of the elevation of serum uric acid concentrations, although it was subsequently demonstrated that this could not be considered a significant toxicity reaction.

Of the 35 toxicity reactions, 21 patients developed hepatocellular dysfunction manifested by abnormal liver function tests. An additional 6 were clinically jaundiced, and 6 others had gastrointestinal symptoms which may have been prodromal symptoms of hepatic disease. Therapy was discontinued for the other 2 because of renal abnormalities; in one patient, an abnormal Addis count and rising blood pressure were noted in the fifth month of treatment. The other patient had advanced bilateral hydronephrosis due to severe, long-standing bilateral caseating renal tuberculosis with nitrogen retention prior to treatment, and developed progressive and fatal uremia following cystoscopy shortly after isoniazid-pyrazinamide was started. It is questionable whether there was any relationship between the renal disease and the drug therapy in these 2 cases.

Treatment was stopped because of toxicity attributed to isoniazid in one patient who received isoniazid-PAS, and in 4 patients who received isoniazid-pyrazinamide. This is not considered a significant difference.

DISCUSSION

In a randomized study in which more than 200 patients were assigned to each of two treatment programs, one of which consisted of isoniazid, 300 mg., and PAS 12.0 gm. daily, and the other isoniazid, 300 mg. and pyrazinamide 3.0 gm. daily, no statistically significant differences could be demonstrated in the therapeutic effectiveness of the two drug regimens, as measured by roentgenographic change, cavity closure, and reversal of infectiousness. Both regimens suppressed the emergence of isoniazid-resistant organisms in large measure. Toxicity to PAS consisted chiefly of gastrointestinal intolerance and hypersensitivity reactions and required change of therapy in approximately 7 per cent of the patients. Toxicity to pyrazinamide presented a more serious problem in 13 per cent of the

patients and consisted chiefly of hepatocellular damage with or without associated jaundice. It is concluded that, while pyrazinamide-isoniazid is an effective drug combination, it does not result in sufficient therapeutic superiority to justify its routine use in the treatment of pulmonary tuberculosis in view of the established significant hepatoxicity of pyrazinamide. Pyrazinamide is recommended for use only in hospitals, where its toxicity is clearly understood and where the patient may be observed daily and regular liver function tests performed; its use should be limited to clinical circumstances in which other equally effective and less toxic drugs are not suitable.

Summary

The results of studies conducted by the Veterans Administration–Armed Forces Conference on the Chemotherapy of Tuberculosis are reported, in which pyrazinamide and isoniazid were evaluated in the treatment of pulmonary tuberculosis. It was observed that, when pyrazinamide was given in a daily dosage of 3.0 gm., it was equally as effective but not superior to para-aminosalicylic acid as a companion drug for use with isoniazid. Hepatotoxicity was a significant hazard from the use of pyrazinamide. This drug is therefore not recommended for use in the initial treatment of active pulmonary tuberculosis.

Sumario

IX. La Piracinamida y la Isoniacida Usadas en el Tratamiento de la Tuberculosis Pulmonar

Preséntanse los resultados de los estudios llevados a cabo por la Conferencia de la Administración de Veteranos y las Fuerzas Armadas sobre la Quimioterapia de la Tuberculosis, en la que se justipreciaron la piracinamida y la isoniacida en el tratamiento de la tuberculosis pulmonar. Se observó que, cuando se administraba la piracinamida a una dosis diaria de 3.0 gms., era igualmente eficaz, pero no superior, al ácido para-aminosalicílico como droga acompañante para uso con la isoniacida. La hepatotoxicidad constituyó un riesgo importante cuando se usó la piracinamida. Por lo tanto, no se recomienda este medicamento para empleo en el tratamiento inicial de la tuberculosis pulmonar activa.

Resume

IX. Utilisation de pyrazinamide et isoniazide dans le traitement de la tuberculose pulmonaire

L'auteur rapporte les résultats d'études faites par l'Administration des Vétérans et les Forces Armées au cours d'une conférence sur la chimiothérapie de la tuberculose. Pendant ces études on a jugé la valeur de la pyrazinamide et de l'isoniazide. On a observé que lorsque la pyrazinamide était donnée à la dose de 3.0 gm journellemente, ce traitement fut aussi actif, mais pas meilleur, que l'acide para-amino-salicylique en tant que médication assoçiée à l'isoniazide. La toxicité vis à vis du foie est un danger à considérer dans l'usage de la pyrazinamide. Ce médicament n'est donc pas recommandé comme traitement initial d'une tuberculose pulmonaire active.

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PLEURAL BIOPSY AND DECORTICATION AS A DIAGNOSTIC TOOL[1]

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INTRODUCTION

It has been axiomatic that pleurisy with effusion of undetermined cause must be considered to be tuberculous in origin and treated accordingly. Other than for whatever onus a diagnosis of tuberculosis might carry, there is no major objection to this approach in practice if it is considered satisfactory to treat the patient on an outpatient basis, with the realization that the antituberculous therapy may be unnecessary, since a significant percentage of idiopathic pleural effusions are not due to tuberculosis. A percentage of error in diagnosis must be expected.

Sutliff, Hughes, and Rice (1) were the first to report on a sizable number of pleural biopsies obtained by thoracotomy for diagnosis. Tuberculosis was the etiologic agent in seventeen of twenty-one cases; three cases were due to carcinoma and one was caused by North American blastomycosis. Those writers concluded that, in the twenty-one to forty age group, operation for diagnosis was not indicated since all of their cases in this category were of tuberculous origin.

Small and Landerman (2) noted that pleural effusion could be caused by a multiplicity of local and systemic conditions and they advocated pleural biopsy. Their five reported cases were all tuberculosis and the histology of the resected pleural membrane indicated an infectious, not an allergic, etiology.

In the same year, Stead, Eichenholz, and Stauss (3) reported on the findings in twenty-four cases of decortication, with a presumptive diagnosis of tuberculous pleural effusion. A ruptured subpleural caseous tuberculous nodule caused the effusion in fifteen of the cases, proving again that the effusion noted in tuberculosis is not due to an allergic response. In seven cases, the pleural membrane was due to trapped lung with free pleural fluid. Fibrothorax from hemorrhage caused the other two pleural membranes.

Breckler and others (4) found, with limited thoracotomy for pleural biopsy, that nine of ten cases thought to be tuberculous were so proved. Other causes for pleural effusion discovered in their series included two intrathoracic neoplasms, two streptococcal pneumonias, and one case each of residual hemothorax and collagen disease.

Twenty-five pleural biopsies were reported by Smith, Parsons, and Daniels (5). *Mycobacterium tuberculosis* was cultured ten times from seventeen specimens having the histologic characteristics of tuberculous infection. One case which yielded a pathologic diagnosis of chronic nonspecific pleuritis subsequently was found to be tuberculous by culture of pleural fluid. False negatives were also encountered by Schless, Harrison, and Wier (6), who believe that this might have been the result of mixed infection or the end result of successful prolonged treatment. This situation seems to be rare.

The recent policy at Valley Forge Army Hospital has been to perform a pleural biopsy in all cases of pleural effusion not diagnosed by other means, including needle biopsy of the pleura. Unfortunately, some of the patients were started on antituberculous therapy on the basis of a positive tuberculin reaction prior to arrival, one patient having received therapy for nine months prior to biopsy. Also, since there were some interesting findings as a result of decortication for fibrous pleural peel thought to be tuberculous, 3 of these cases will be included in this report.

MATERIALS AND METHODS

A majority of those who have discussed limited thoracotomy for pleural biopsy describe a procedure performed under local anesthesia. The present writers have elected endotracheal anesthesia since it is then possible to palpate or biopsy the lung if it is deemed necessary. The procedure has been performed through an intercostal approach as well as through the periosteal bed of a portion of resected rib. The latter method gives better exposure and is the method of choice. The site of incision is governed by the area of disease, but is generally in the eighth rib area laterally, which makes available the costophrenic sulcus and edge of the lower lobe. There have been no complications, and morbidity has been minimal.

[1] From Valley Forge Army Hospital, Phoenixville, Pennsylvania.

Observations

Fifteen pleural biopsies for diagnosis have been performed in the past fourteen months. Six of these have revealed a granulomatous process compatible with tuberculosis. *Mycobacterium tuberculosis* was isolated in 5 of these cases by culture or guinea pig inoculation. The remaining 9 patients were diagnosed as having chronic nonspecific pleuritis on the basis of the pleural biopsy. These 9 patients were persons who had never been treated as well as some who had received up to nine months of antituberculous therapy. It is possible that, as shown by others (5, 6), a diagnosis of tuberculosis may have been missed in patients who had received long-term chemotherapy.

The 3 patients whose decortication procedures were selected for inclusion were operated on for fibrothorax after a presumptive diagnosis of tuberculous pleural effusion and months of chemotherapy. They are presented as case reports.

Case Reports

Case 1. A 44-year-old male had an exploratory laparotomy in Germany for severe abdominal pain. The pancreas was biopsied, acute pancreatitis diagnosed, and he soon returned to duty in Greece. Several months later the patient developed right pleural effusion. Thoracenteses were performed in Germany; he was started on chemotherapy for tuberculosis and was transferred to Valley Forge Army Hospital. Decortication three months later resulted in a diagnosis of metastatic anaplastic carcinoma. Eventually, autopsy revealed that the probable site of origin was in the pancreas. Figure 1 is the preoperative chest roentgenogram.

The diagnosis was missed until the histologic sections of the resected specimen were available.

Case 2. A 22-year-old soldier was hospitalized because of weight loss, lassitude, and roentgenographic evidence of left pleural effusion. On the basis of a positive tuberculin reaction, he was started on antituberculous chemotherapy and was evacuated from Korea. This course resulted in no change in the roentgenographic appearance. It was believed that the patient had a thick peel with a trapped lung. At surgery he was found to have a thick parietal peel, a huge space containing about 300 ml. of clear yellow fluid, and a totally atelectatic lung. The lung measured no more than 10 cm. in greatest diameter and was covered with a thick peel. Following decortication and days of

Fig. 1. Decortication revealed the diagnosis to be metastatic anaplastic carcinoma.

negative pressure suction, the lung re-filled the hemithorax. There was no evidence of tuberculosis.

In retrospect, this patient is believed to have had a total spontaneous pneumothorax which did not re-expand. Pleural fluid formed, stimulating the formation of fibrous tissue on visceral and parietal pleurae. This created the roentgenographic picture which caused his hospitalization. Even in retrospect, the roentgenograms shown in figure 2 defy proper interpretation.

Case 3. During basic training a 26-year-old soldier was hospitalized and treated for pneumonia. Shortly thereafter he was transferred to Europe, where he was soon hospitalized with fever, weight loss, lassitude, and cough. A roentgenogram revealed a diffuse chest opacity. Thoracentesis was unsuccessful, and the patient was evacuated to this hospital while receiving antituberculous chemotherapy. Medication was continued but he remained febrile, the roentgenographic picture did not change significantly, and tuberculosis could not be proved. Decortication was undertaken for diagnosis and treatment. A very thick, adherent peel was encountered. In the course of the procedure, an abscess was broken into. From this abscess, hemolytic *Staphylococcus aureus* was cultured. He developed a staphylococcal wound infection but eventually had a successful outcome and returned to full duty. The preoperative and postoperative roentgenographic appearance is shown in figure 3.

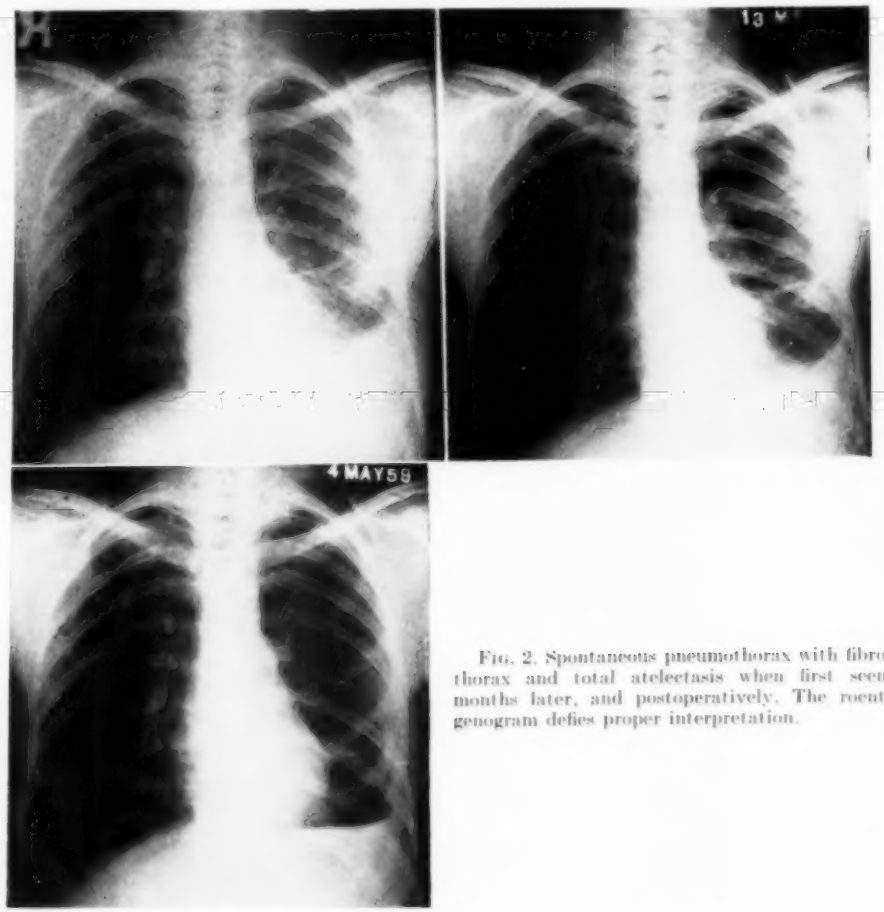

FIG. 2. Spontaneous pneumothorax with fibrothorax and total atelectasis when first seen, months later, and postoperatively. The roentgenogram defies proper interpretation.

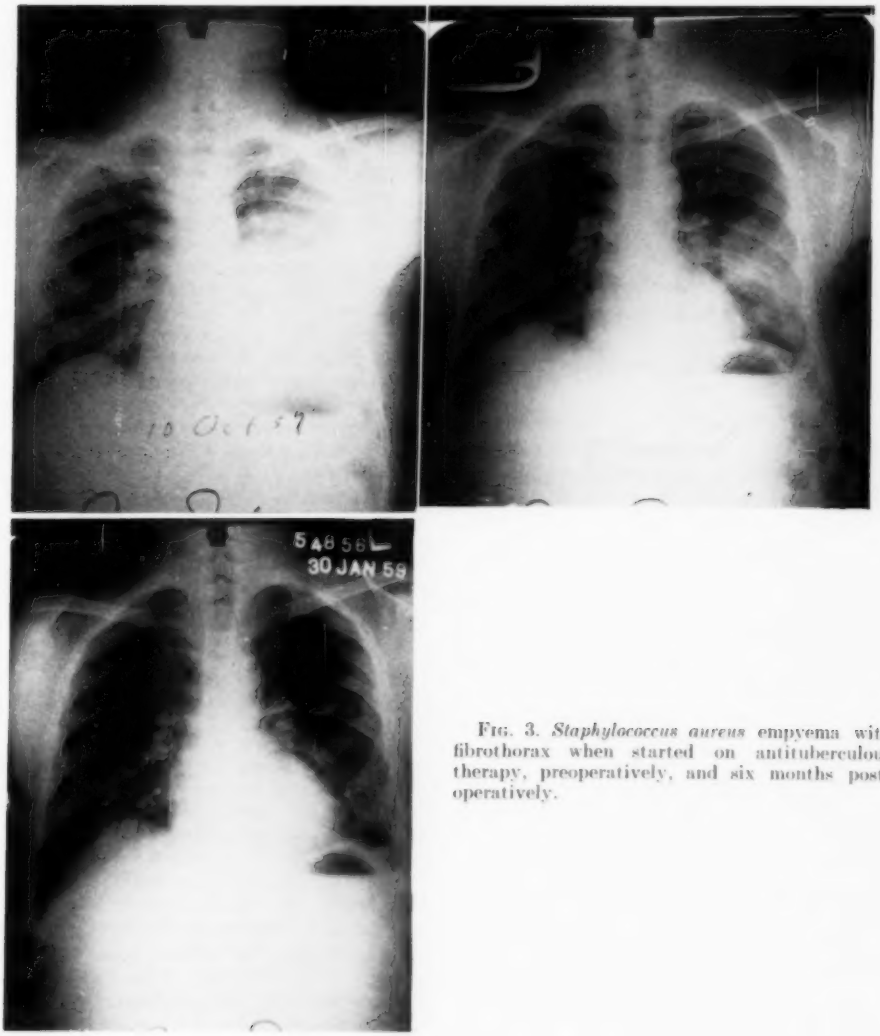

FIG. 3. *Staphylococcus aureus* empyema with fibrothorax when started on antituberculous therapy, preoperatively, and six months postoperatively.

DISCUSSION

The assumption that pleural effusion of undetermined etiology is of tuberculous origin is not tenable in view of the results obtained by pleural biopsy. While the reports of various investigators on small series of cases show a percentage difference in etiologies, the trend is the same in each case. A significant portion of these patients are not tuberculous and need not be treated as such. Following the principle of not permitting any pleural effusion to remain an etiologic enigma, it has been shown in the present study that a significant proportion of patients are not tuberculous even in a young population such as is encountered in the Army.

An aggressive approach in search of a definite diagnosis is important to the Army, not only from the standpoint of good medicine, but

because a great deal of money is involved. Tuberculosis cannot be treated on a duty status and can lead to at least temporary retirement. A diagnosis of nonspecific pleuritis permits rapid return of the individual to duty. At a cost of approximately $20 a day for hospitalization, plus the individual's pay, which he receives even though not gainfully employed, the economic facts are obvious. It is disturbing to think of the amount of money that has been and is being paid out in pensions and other veterans' benefits to persons who have been separated from military service with histologically or bacteriologically unproved diagnoses of tuberculous pleural effusion.

A majority of the patients in this hospital who were diagnosed as having chronic pleuritis gave histories of preceding acute pulmonary infections followed by pleural effusion.

On the basis of the writers' experience with this group of cases, it has been concluded that it is obligatory to perform a pleural biopsy by limited thoracotomy if lesser measures to include culture of fluid obtained by thoracentesis or needle biopsy of the pleura fail to yield an etiologic diagnosis in pleural effusion.

Summary

Pleural biopsy by limited thoracotomy in 15 cases of pleural effusion of undetermined etiology revealed 6 cases of granulomatous pleuritis compatible with tuberculosis. The specific organism was obtained in 5 of these cases by culture or guinea pig inoculation. The remaining 9 cases revealed no evidence of tuberculosis. It is conceded that the diagnosis might be missed in patients who have received long-term chemotherapy prior to pleural biopsy.

Three decortications, presumably for organized tuberculous pleural effusion, revealed other etiologies to include metastatic carcinoma, *Staphylococcus aureus* empyema, and trapped lung secondary to spontaneous pneumothorax.

Pleural biopsy by limited thoracotomy is recommended for diagnosis if simpler procedures fail to lead to a definitive diagnosis in pleural effusion. The procedure is valuable from both the diagnostic and economic standpoint.

Sumario

La Biopsia y la Decorticación Pleural como Instrumento de Diagnóstico

La biopsia pleural, por medio de una toracotomía limitada, en 15 casos de derrame pleural de etiología indeterminada, reveló 6 casos de pleuritis granulomatosa compatible con tuberculosis. En 5 de estos casos se obtuvo el microbio específico por el cultivo o la inoculación en el cobayo. Los otros 9 enfermos no revelaron signos de tuberculosis. Se concede que el diagnóstico puede fallar en enfermos que han recibido quimioterapia a largo plazo antes de la biopsia pleural.

Tres decorticaciones, presuntamente por derrame pleural tuberculoso organizado, revelaron otras etiologías que comprendieron metástasis carcinomatosa, *Staphylococcus aureus*, empiema y retención del pulmón secundaria a neumotórax espontáneo.

Se recomienda para el diagnóstico la biopsia pleural por medio de una toracotomía limitada si procedimientos más sencillos no conducen al diagnóstico definitivo en el derrame pleural. El procedimiento es valioso desde el punto de vista tanto diagnóstico como económico.

Resume

Biopsie pleurale et décortication, procédés de diagnostic

Une biopsie pleurale par thoracotomie localisée dans 15 cas d'épanchement pleural, d'étiologie non définie, a montré dans 6 cas une pleurite granulomateuse compatible avec une tuberculose. L'organisme spécifique fut mis en évidence dans 5 de ces cas par culture ou inoculation au cobaye. Il fut impossible de faire la preuve de la tuberculose chez les 9 autres malades. Il est admis que le diagnostic peut être difficile à établir chez les malades qui ont été soumis à une longue chimiothérapie avant la biopsie pleurale.

Trois décortications, pour épanchement pleural organisé et supposé tuberculeux, ont révélé des étiologies différentes, telles qu'un carcinome métastatique, le staphylocoque doré, un empyème avec pincement pulmonaire secondaire à un pneumothorax spontané.

La biopsie pleurale par thoracotomie localisée est recommandée comme méthode de diagnostic, si des méthodes plus simples n'ont pas permis de diagnostic dans un épanchement pleural. Cette méthode est satisfaisante du point de vue diagnostique et économique.

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THE FINDING OF GIANT CELLS IN THE SPUTUM OF TUBERCULOUS PATIENTS[1]

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Introduction

It has been shown in recent years that giant cells in atypical situations are commonly seen in pulmonary tuberculosis treated with antimicrobial drugs (1–3). Loose giant cells may be encountered within alveolar spaces and evanescent granulomata, as well as within bronchioles and at the surface of healing tuberculous cavities. They can be expectorated and found in the sputum (4). Some facts suggest that the elimination of giant cells by the bronchial route is chiefly connected with the loss or attenuation of the pathogenicity of tubercle bacilli (5). In any event, the finding of giant cells in the sputum of tuberculous patients would ultimately result in the relatively easy destruction and elimination of tubercle bacilli and their chemical debris. This explanation would be in line with the lung tissue reaction against the inoculation by intravenous or intratracheal route of inert or living particles. Many years ago, Leuret and Caussimon (6) verified that everything happened as if the lung tried to eliminate the inert, toxic, or virulent particles from the septa to the alveoli and the bronchial tree. The reaction pattern was always the same, but the case of alveolar elimination was inversely proportional to the toxicity or virulence of the particles. The present paper is an elaboration of the previously reported finding of giant cells in the sputum raised by tuberculous patients treated with antimicrobial drugs (4).

Material and Methods

Samples of sputum from 131 hospital patients were searched for giant cells. The sputum raised by the patients was collected in flasks containing a mixture of ethanol and ether (1:1). After embedding the samples in paraffin, microscopic sections were made as usual and stained with hematoxylin and eosin. All patients had advanced pulmonary tuberculosis routinely treated with antimicrobial drugs. The drug regimen consisted of daily isoniazid associated with streptomycin every third day or with daily PAS. Most patients had already been treated with chemotherapeutic agents before admission.

The sputum of 105 patients was positive for tubercle bacilli either by direct examination or, in a few cases, by culture. In 21 cases no tubercle bacilli were found in repeated sputum smears or by sputum culture at the time of the search for giant cells. In the remaining 5 cases no recent data on sputum examination were available. Susceptibility tests were performed in 55 of the 105 patients whose sputum was positive for tubercle bacilli. In 38 of these 55 cases the tubercle bacilli were resistant to a minimal concentration of 0.2γ of isoniazid and 2γ of streptomycin per ml. of medium; in 13 and in 4 cases, respectively, tubercle bacilli were susceptible to streptomycin alone or to streptomycin and isoniazid.

Results

Giant cells were found in 20 cases (15.2 per cent) (Plates I and II). In 7 cases only one giant cell could be found; in 6 cases, 2; in 4 cases, 3; in one case, 5; and in 2 cases, 6. As a rule, only one or two microscopic sections were thoroughly searched for giant cells in each case. Therefore, the percentage of cases with giant cells in the sputum might have been substantially larger if serial sections had been examined. The width of the giant cells found in the sputum of the patients ranged all the way from 20 to 70 micra. They contained 4 to 20 nuclei or more. In the great majority of the cases, the nuclei were peripherally arranged. Foreign-body giant cells occurred in 3 cases. In general, the giant cells were small (averaged about 35 micra) and contained a few peripheral nuclei (averaged about 8). Large lipoid inclusions and phagocytosed polymorphonuclear leukocytes occurred in 4 and 6 cases, respectively. Drug-susceptibility tests were carried out in only 6 of the 20 cases with giant cells in the sputum. In all of these 6 cases the tubercle bacilli were resistant to isoniazid. The correlation between the duration of previous chemotherapy and the finding of giant cells in the sputum is shown in table 1.

Discussion

The finding of giant cells in the sputum raised by tuberculous patients could be predicted after they were seen existing freely in the tissue, completely unattached within alveolar spaces and

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bronchioles. The phenomenon seems to be related to the action of antimicrobial drugs, since these drugs are clearly responsible for the atypical histologic aspects of pulmonary tuberculosis reported in recent years (1–5). Giant cells have been encountered in secretions bronchoscopically aspirated from tuberculous patients (7, 8) but, as far as is known, not in the sputum coughed up spontaneously. In the present material, the number of patients treated during a few days or weeks is too small. However, giant cells were found in the sputum of 3 patients within the first month of treatment. In 2 of them this finding coincided temporally with rapid liquefaction of caseous lesions. On the other hand, pneumonic consolidations characterized by the filling of alveoli with epithelioid cells and giant cells were already observed in humans before the antimicrobial era. For instance, the so-called epituberculosis was sometimes an epithelioid and giant cell pneumonia, which, as a rule, cleared gradually in serial roentgenograms. It seems that, in such lesions, loose giant cells were incidentally found within alveolar spaces (9). Nevertheless, its relative infrequency and benign character should explain the absence of observations on giant cells in atypical situations and in the sputum raised by tuberculous patients before the routine use of pulmonary resection and antimicrobial drugs in the treatment of tuberculosis.

It is interesting to note that an identical histologic picture is seen in guinea pigs inoculated with massive doses of isoniazid-resistant strains of low pathogenicity. The elimination of giant cells by the bronchial route may be clearly observed in such experimental pneumonias (5). Thus, different combinations of the factors responsible for the characteristics of tuberculous lesions may give rise to the same histologic picture, in which the absence of necrosis and the tendency to complete resolution are important features.

These observations do not warrant the conclusion that the finding of giant cells in the sputum is more probable in case of prolonged chemotherapy, i. e., extending for more than six months, since the reported differences are not statistically significant. No definite statement can be made in this respect, unless the elimination of giant cells in the sputum is studied in two comparable groups of patients eliminating "wild type" or drug-resistant bacilli. However, recent data show that about 70 per cent of the patients with infectious sputum have strains simultaneously resistant to isoniazid and streptomycin at the time of admission to this hospital. The great majority of these patients have already been treated before admission, sometimes very irregularly.

Resistance to both drugs occurred in 38 of the 55 cases (69 per cent) reported above, in which drug-susceptibility tests were available. This percentage goes up to 92.7 if only isoniazid resistance is taken into consideration. Moreover, the simultaneous presence of giant cells and tubercle bacilli in the sputum was the most frequent alternative. There were giant cells in the sputum of 17 of the 105 patients with infectious sputum and in 2 of the 21 patients with noninfectious sputum. Although the differences are not significant, they seemingly express the fact that, as a rule, the chances of finding giant cells in the sputum are greater in chronic drug-resistant patients than in successfully treated patients. Altogether, these observations are consistent with the hypothesis that the giant cells found in the sputum of tuberculous patients treated with antimicrobial drugs originate chiefly from atypical regressive lesions. The same phenomenon might have been observed before the antimicrobial era, but it is highly probable that its frequency has increased substantially, and that such increase is associated, in the main, with the destruction of isoniazid-resistant bacilli of low pathogenicity.

Another interesting point is the small size of most giant cells encountered in the sputum produced by tuberculous patients. The presence of small giant cells with a few nuclei had already been registered by Coulaud in his classic work on experimental infection with BCG (10). As mentioned above, the same aspect is commonly seen in the experimental tuberculosis of guinea pigs inoculated with isoniazid-resistant strains of low pathogenicity (5). Small giant cells are also produced by injecting guinea pigs with large doses of lipopolysaccharide of tubercle bacilli (11). While giant cells with a few peripheral nuclei are found in untreated human tuberculosis, it is the impression of the present writers that, after the use of antimicrobial drugs, an increase of these particular cellular elements has accompanied the total increase of specific cellular metaplasias, as Canetti suggests (12). The known data indicate that the formation of epithelioid and giant cells

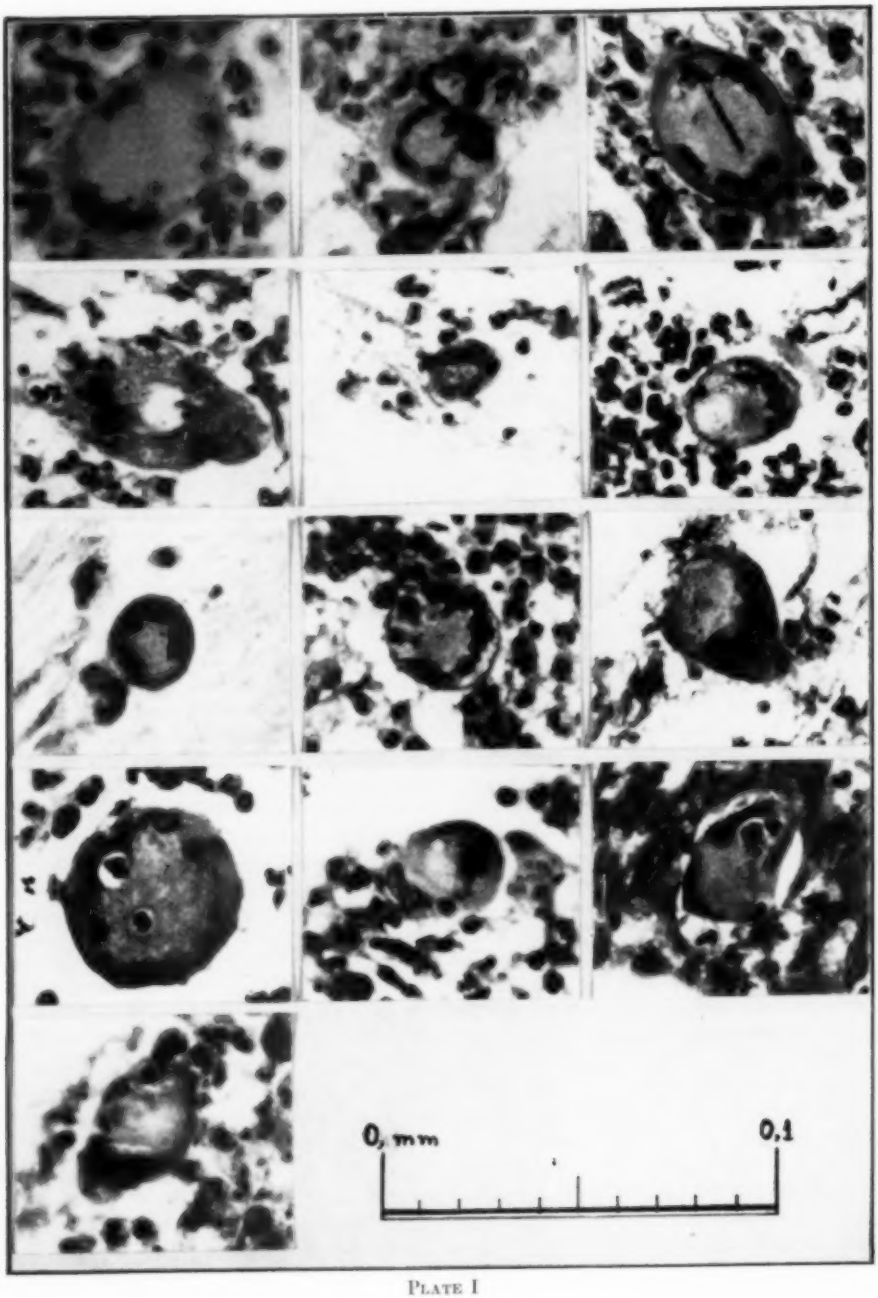

PLATE I

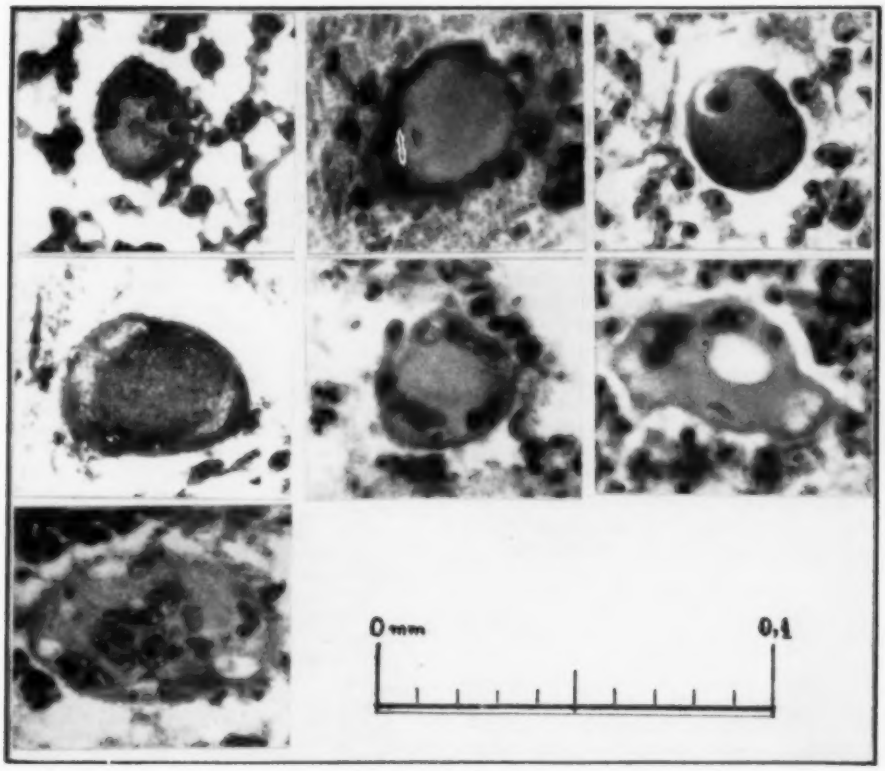

PLATE II

PLATES I AND II

Giant cells found in the sputum of 20 tuberculous patients treated with antimicrobial drugs.

TABLE 1

PRESENCE OF GIANT CELLS IN THE SPUTUM OF 131 TUBERCULOUS PATIENTS RELATED TO THE DURATION OF PREVIOUS CHEMOTHERAPY

Duration of Previous Chemotherapy	Giant Cells in the Sputum		Total
	+	−	
months			
0–1	3	20	23
1–6	3	23	26
6–12	3	21	24
More than 1 year	11	47	58
Total	20	111	131

follows the death and disintegration of tubercle bacilli within the macrophages (13–15). The specific cellular metaplasias seem to be the result of the irritating action of tuberculolipids upon the mononuclear phagocytes (12, 15, 16).

It is true, as Rich stressed, that experimental injections of lipid fractions have not uncovered what it is in the tubercle bacilli that produces tubercles in the tuberculous body (17). However, it is highly probable that such power is related to some lipid derivative of the tubercle bacilli and that the type of specific cellular metaplasias occurring in the tuberculous body depends on the chemical nature of the substances, their molecular weight, their physical properties, and their total amount (18). Differences in chemical composition, particularly with regard to the total amount of lipid fractions, have been reported between virulent and avirulent tubercle bacilli, as well as between "wild" and isoniazid-treated bacilli (19, 20). Therefore, an acceptable

working hypothesis would be that the relatively frequent finding of small giant cells in the sputum raised by tuberculous patients treated with antimicrobial drugs is due to a deeper cleavage of lipid fractions originating from tubercle bacilli of low pathogenicity.

Summary

The writers reported the finding of giant cells in the sputum raised by 20 of 131 tuberculous patients treated with antimicrobial drugs. Expectoration of giant cells could be predicted after they were seen completely loose within alveolar spaces and bronchioles. Such a phenomenon may be observed in the early stage of chemotherapy and there is some reason to believe that it might have occurred to a very limited extent before the antimicrobial era. However, the available facts strongly suggest that the elimination of giant cells in the sputum of tuberculous patients is now chiefly connected with the destruction of isoniazid-resistant bacilli of low pathogenicity.

Sumario

El Hallazgo de Células Gigantes en el Esputo de los Tuberculosos

Describese el hallazgo de células gigantes en el esputo expulsado por 20 de 131 tuberculosos tratados con medicamentos antimicrobianos. Cabía predecir la expectoración de tales células después de observarlas absolutamente desprendidas dentro de los espacios alveolares y los bronquiolos. Puede observarse este fenómeno en la fase incipiente de la quimioterapia y hay algunos motivos para creer que hubiera podido registrarse ya antes de la era antimicrobiana. No obstante, los datos disponibles sugieren elocuentemente que la eliminación de células gigantes en el esputo de los tuberculosos se encuentra hoy día enlazado principalmente con la destrucción de bacilos isoniacidorresistentes de poca patogenicidad.

Resume

Découverte de cellules géantes dans l'expectoration de tuberculeux

Les auteurs rapportent la découverte de cellules géantes dans l'expectoration de 20 des 131 malades traités par des agents antimicrobiens. Des cellules géantes ont pu être décelées libres dans les espaces alvéolaires et les bronchioles. Un tel phénomène peut être observé précocément au cours du traitement et on a quelque raison de croire qu'il aurait pu être observé avant l'ère des antibiotiques. Cependant, les faits actuels font penser que l'élimination de cellules géantes dans les crachats de tuberculeux est plutôt en relation avec la destruction de bacilles ayant un faible pouvoir pathogène et étant résistant à l'isoniazide.

Acknowledgment

The writers wish to express their sincere appreciation for the collaboration of Dr. Francisco Rosado Filho and Dr. Eteocles Magnavita.

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THE QUANTITATIVE DETERMINATION OF SMALL AMOUNTS OF ISONIAZID IN BODY FLUIDS USING A CATIONIC EXCHANGE RESIN[1, 2]

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INTRODUCTION

Although the mechanism of the tuberculostatic action of isoniazid is not clear (1), it is well recognized that the drug is effective only in the free form. The metabolites of isoniazid are generally without bacteriostatic effect with the possible exception of the hydrazones alpha-ketoglutaric acid isonicotinoyl hydrazone and pyruvic acid isonicotinoyl hydrazone (2), which may be cleaved by hydrolysis *in vivo* to yield free isoniazid (3). The maintenance of free-isoniazid drug concentrations as well as the rate of metabolic inactivation seems to be an individual variable (2, 4). Consequently, investigators are increasingly aware of the fact that research directed toward a more rational therapeutic utilization of this potent tuberculostatic drug depends partially upon the development of a sensitive, reliable, and simple chemical method for the determination of free isoniazid.

In response to this need, a procedure has been developed in this laboratory that is believed to represent a practicable method for the determination of free isoniazid and acetyl-isoniazid. With this test, reliable measurements have been made of concentrations as low as 0.1 γ of isoniazid or isoniazid derivative per ml. The ability to measure concentrations of less than 1 γ per ml. is required for the determination of free isoniazid in serum withdrawn six hours or more after the patient ingests 2.5 mg. of isoniazid per kg. of body weight. Measurements in this range are also required for determining drug concentrations in serum after much higher dosages when the total serum concentration is comprised largely of acetyl-isoniazid. Other chemical methods investigated by this laboratory (2, 5-9) do not achieve sensitivity of this magnitude, the maximal sensitivity being 1 γ per ml., and values below this must generally be extrapolated. In addition, some of these tests are not specific, as they do not distinguish between free isoniazid and its acetylated derivative, thus placing limitations on the usefulness of these tests.

The widely used microbiologic assay method of Morse and associates (10), which measures the antimicrobial properties of serum from patients treated with isoniazid, has a disadvantage in that it is time consuming since it depends upon the growth of *M. tuberculosis*. In addition, it is generally recognized that all microbiologic methods have gross inherent inaccuracies.

In the course of a study of chemical methods for the determination of isoniazid, the attention of the writers was drawn to a recent paper by Dr. Walter Nielsch (11), who reported an extremely sensitive and specific test for isonicotinic acid. This reaction is based on the combination of isonicotinic acid with chloramine-T and barbituric acid in the presence of an excess of cyanide ion and acetone, whereupon an intense blue color is produced.

It has been possible to confirm the sensitivity and specificity of this reaction for isonicotinic acid and for other pyridine-4-derivatives after acid hydrolysis. It is, therefore, the purpose of this paper to report a modification of the analytical color technique of Nielsch (11) and its use in conjunction with a cationic resin for the separation and determination of isoniazid and acetyl-isoniazid in biologic material.

METHOD

Reagents:

Acetone (reagent grade).

Acetic acid, 1.0 N.

Potassium cyanide, 10 per cent. This solution was prepared daily and refrigerated until use.

Chloramine-T (sodium *p*-toluenesulfonchloramide), 10 per cent. This solution was kept at refrigerator temperature.

Acetyl-isoniazid elution mixture. This mixture was prepared so as to obtain a solution having a final concentration of 0.20 molar lithium chloride, 0.10 molar potassium chloride, 0.10 N hydrochloric acid, all in 10 per cent ethyl alcohol V/V.

Isoniazid elution reagent. 6 N hydrochloric acid (reagent grade).

[1] From Leahi Hospital, Honolulu, Hawaii.

[2] This study was aided by a Ford Foundation grant.

Ion exchange resin (cationic).[2] Ion X, Dowex 50×8[4] $[H^+]$ form. (Mesh size 200 to 400, 50×8 cross linked.) This resin was converted to the free base form with 1 N sodium hydroxide, washed thoroughly till neutral with distilled water, then converted to the $[H^+]$ form using first 2 N then 1 N hydrochloric acid. After conversion to the $[H^+]$ form, the resin was washed with distilled water until a reaction of pH 7.0 was obtained on the wash water. The resin was stored and used in the wet form. (*Note:* Blank exchange columns should be prepared and treated as in the method, using 0.85 per cent sodium chloride in the place of serum. If the blank columns give excessive background with the color reagent, the resin should be rewashed until the background is completely removed).

Acetate buffer (exactly pH 5.2). Composition of this buffer was as follows:

Solution A: 229 ml. of glacial acetic acid diluted to one liter.

Solution B: To a beaker containing 544 gm. of sodium acetate (CH_3-$COONa$-$3H_2O$) 500 ml. of distilled water were added, and the mixture was carefully heated until solution was complete. The contents of the beaker was then transferred to a one-liter volumetric flask, cooled, and diluted to volume.

The acetate buffer solution was prepared by mixing 295 ml. of solution "A" with 705 ml. of solution "B." This buffer must have a reaction of pH 5.2. To check the pH, the prepared buffer was diluted 1:20 prior to reading on a pH meter.

Barbituric acid solution, 2 per cent (pH 5.2). Twenty grams of barbituric acid (Eastman), recrystallized four times from water and dried, were placed in a beaker containing 750 ml. of distilled water. Sodium hydroxide 5 N was added dropwise from a buret with constant stirring without heat, until a stable reaction of pH 5.2 was obtained, i.e., the pH of the solution did not drop below pH 5.2 on continued stirring. The supernatant solution was transferred to a one-liter volumetric flask. To the undissolved barbituric acid remaining in the beaker 200 ml. of distilled water were added. Stirring and adjustment to pH 5.2 were carried out as above until all of the barbituric acid was dissolved. This solution was added to that in the one-liter flask, and the combined solutions were diluted to volume with distilled water. An occasional turbidity which developed in the diluted reagent was removed by filtration through acid-washed Whatman No. 1 filter paper.

Mixed color reagent. To 5 ml. of 10 per cent chloramine-T was added exactly 1.0 ml. of fresh 10 per cent potassium cyanide. Then 1.0 N acetic acid was added, with stirring, from a 5-ml. buret until a reaction of pH 5.2 was obtained. The amount of acetic acid used was recorded.

Into a 125-ml. glass-stoppered Erlenmeyer flask were placed 6.25 ml. of acetate buffer (reagent 8) followed by 1.0 ml. of cold, 10 per cent potassium cyanide solution with mixing. To this, 5 ml. of 10 per cent chloramine-T were added, with gentle swirling. (This produces a marked precipitate.) In rapid order the recorded amount of 1.0 N acetic acid (1.4 to 2 ml.) and 5 ml. of acetone were added. At this point the solution was clear. The solution was mixed by swirling, and 12.5 ml. of 2 per cent barbituric acid solution were added. The completed reagent was mixed and placed in the dark for three minutes before use.

Preparation of the resin column: Kolmer graduated centrifuge tubes,[5] 10 ml., were prepared by heating the tip in a burner flame and passing a long hypodermic needle (20-gauge) down the inside of the tube and through the softened tip, producing a small protrusion with a tiny hole. A small whisp of glass wool was placed in the tube tips to retain the resin. Resin slurry was then added to the tube until the volume of the resin was 0.7 ml. A small disc of acid-washed filter paper which had the same diameter as the constricted portion of the tube was placed on the upper surface of the resin. This was to ensure a flat upper column surface and to prevent leakage of the resin during the packing process. On top of the disc of filter paper a tight plug of glass wool was tamped. The column was washed with 5 ml. of 6 N hydrochloric acid followed by 5 ml. of distilled water. This process was repeated three times. As the acid was added, shrinkage of approximately 0.1 ml. occurred. At this point the glass wool and paper disc were tamped down tightly to the new level. The column was considered to be adequately packed when expansion of the resin did not occur during the addition of water and the resin volume remained at 0.6 ml. Finally, the column was washed with distilled water until the effluent was neutral (pH 7.0). These columns, after packing, may be stoppered and kept ready for use. The columns will keep indefinitely when prepared in this manner.

Under no circumstances was the column allowed to run dry, i.e., during use and storage the liquid level in the tube was always kept above the level of the upper glass wool plug.

Test Procedure

Column separation of isoniazid and acetylisoniazid: To the washed column prepared as above 1 to 3 ml. of serum were added. The serum was forced through the column, using laboratory line air pressure, at a flow rate of approximately 0.5 ml. per minute until the liquid level just touched the top of the glass wool plug. The column

[2] Obtained from Microchemical Specialties Co., 1834 University Avenue, Berkeley 3, California.

[4] Batch No. L 3966/14.

[5] Corning Item No. 8360, catalogue No. LG-1, Corning Glass Works, Corning, New York.

was then washed three times with 5-ml. portions of distilled water. These washings were discarded. Three 5-ml. portions of the acetyl-isoniazid elution mixture were then forced down the column at the same flow rate as above. These three washes were collected in a 60-ml. glass-stoppered weighing bottle labeled "acetyl-isoniazid," and 1 ml. of concentrated hydrochloric acid (reagent grade) was added. Two 5-ml. washes of 6 N hydrochloric acid were then forced through the column as above and collected in another glass-stoppered weighing bottle labeled "isoniazid." Between the two acid washes and following the last acid wash 3 ml. of distilled water were added. The water washes were also collected in the same bottle labeled "isoniazid." Both bottles were then placed in a sand bath, set so as not to exceed 160°C., and the contents were evaporated to dryness.

Acid hydrolysis: Four milliliters of 4.8 N hydrochloric acid were added to each bottle. The bottles were stoppered and placed in an autoclave for fifteen minutes at 121°C. (15-pound pressure). The bottles were removed from the autoclave, placed in the sand bath, and the contents were evaporated to dryness. Heating was continued until no traces of hydrochloric acid could be detected.

Color development: From a 3-ml. volumetric pipette, 3 ml. of mixed color reagent were added to each weighing bottle with constant swirling. Swirling of the bottle after stoppering was continued until all of the salt residue was dissolved. The bottles were then allowed to stand in the dark at room temperature for thirty minutes for complete color development.

The colored solutions were transferred to 1-cm. quartz cuvettes and read at 595 millimicrons in a Beckman model B spectrophotometer using mixed color reagent as reference. The unknown values were then calculated from previously prepared acetyl-isoniazid and isoniazid optical density vs. concentration curves. Curves were prepared from hydrolyzed isoniazid and acetyl-isoniazid standards in the range of 0.1 to 1 γ of the respective compound per ml. Alternatively, an isonicotinic acid curve may be prepared in terms of micromolar concentration and the micromolar values calculated for the respective molecular weight of each compound, viz., isoniazid and acetyl-isoniazid.

Results

The absorption spectra are shown in figure 1 for three concentrations of the isonicotinic acid color complex, viz., 0.2, 0.5, and 0.8 γ of isonicotinic acid per ml. in 3 ml. of color reagent.

Because of the extremely narrow absorption band of this peak, as may be seen from the resultant curves in figure 1, the exact point of maximal density must be determined for the particular instrument and reagents which are to

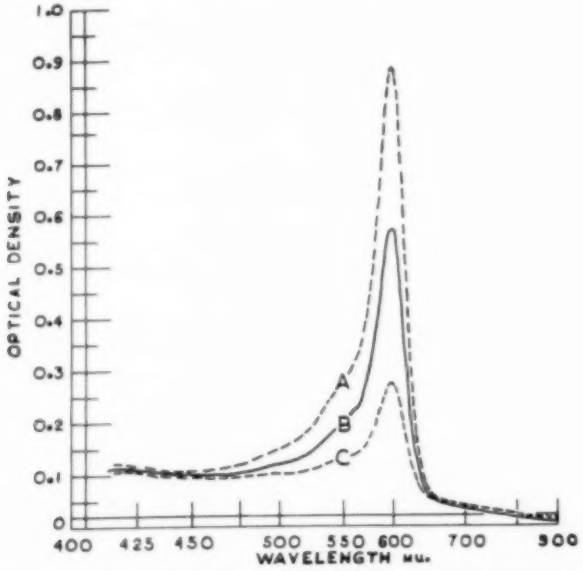

Fig. 1. Extinction curves obtained with three concentrations of isonicotinic acid complexed with color reagent. Readings were made in 1-cm. cuvettes placed in a Beckman DK-2 spectrophotometer. Curve values in γ per ml.: A—0.8, B—0.5, C—0.2.

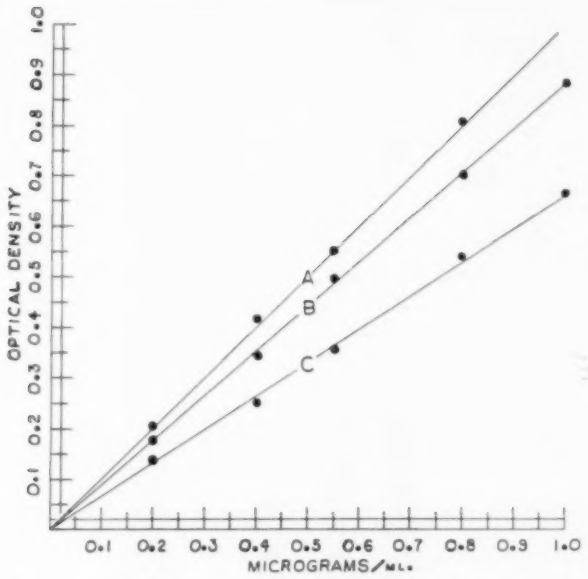

FIG. 2. Bouger-Beer curves for the color complex developed with isonicotinic acid, isoniazid, and acetyl-isoniazid, carried through the entire test procedure. A—isonicotinic acid; B—isoniazid; C—acetyl-isoniazid.

be used. It is apparent that optical density values will fall off rapidly if the exact point of maximal density is not used. For example, as may be seen in figure 1, the point of maximal optical density for the DK-2 ratio recording spectrophotometer was 600 millimicrons; the actual observed and used maximum for the Beckman model B spectrophotometer was 595 millimicrons. While the use of a broad band pass colorimeter would eliminate this problem, some sacrifice in sensitivity would result. The extreme sensitivity of the presented test will of course permit the use of a broad band pass. As indicated above, however, if an instrument such as the Beckman model B is to be used at narrow slit openings, careful wave-length selection is required for maximal resolution. Quartz cuvettes were used in the present experiments, but are not essential.

Presented in figure 2 are the Bouger-Beer curves prepared from isonicotinic acid, isoniazid,[6] and acetyl-isoniazid,[7] in serum ranging from 0.1 to 1 γ per milliliter. These substances were carried through the entire test procedure. Each point in figure 2 represents the results of five separate determinations.

The linearity of these curves reflects the conformance with Beer's Law of these compounds under the conditions described. The sensitivity shown in these curves has proved to be more than adequate for all drug concentrations of isoniazid and acetyl-isoniazid which the writers have encountered thus far. Indeed, as much as a sixfold dilution was at times required in order to bring the optical density of the final solution within a readable range of the spectrophotometer. The quantity of biologic sample used for a test varied according to anticipated drug concentrations. For serum samples taken two to six hours after ingestion of 2.5 mg. per kg. or less, 1 to 3 ml. of sample usually were required. This placed most values in the readable range.

To determine the efficiency of the Dowex 50 $\times$ 8 resin column in extracting and separating isoniazid and acetyl-isoniazid in serum and in aqueous solutions, an aqueous solution and a solution of drug-free pooled serum were prepared, each containing 1.0 γ of isoniazid per ml., and 1.0 γ of acetyl-isoniazid per ml. Three milliliters

[6] Isoniazid Squibb No. 7425, Lot No. 24210-045.

[7] Acetyl-isoniazid Squibb No. 7787, batch 2. Obtained through the courtesy of E. R. Squibb & Sons, New Brunswick, New Jersey.

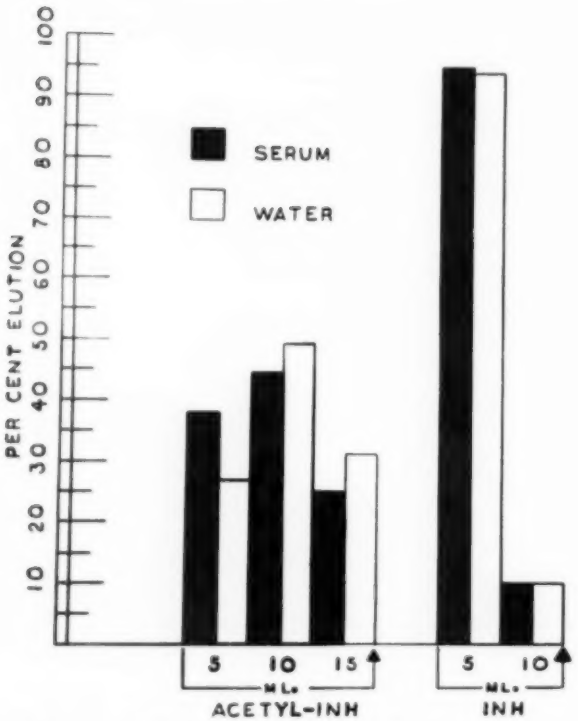

FIG. 3. Per cent elution vs. milliliter of eluting solution. One microgram of isoniazid and acetyl isoniazid per ml. of serum and distilled water. Elution from Dowex-50 × 8.

of each of these solutions were carried through the entire test procedure as presented in this paper; however, each 5-ml. volume of eluting reagent was collected separately and was analyzed to determine the percentage of isoniazid and acetyl-isoniazid which was eluted by each fraction.

The bar graph in figure 3 shows the results of this experiment. It was noted with great interest that there was a distinct difference between water and serum solutions in the elution pattern of acetyl-isoniazid. However, clear-cut separation of isoniazid and acetyl-isoniazid was achieved with both serum and water.

Although not included in the text of this paper, many differing acid, salt, and alcohol concentrations were tried as eluting solutions; however, the acid, salt, and alcohol concentrations given in the text were found to be the optimal combination for consistent, clear-cut separation of the two compounds.

As far as could be detected, with this combination of resin and salts, none of the added isoniazid occurred in the acetyl-isoniazid elution fraction. Repeated duplication of this work using varying amounts of acetyl-isoniazid and isoniazid ranging from 1,000 γ per ml. to 0.2 γ per ml. has shown that all of the acetyl-isoniazid is selectively eluted well within the indicated 15 ml. of eluting fluid. In fact, by further fractional eluting of the last 5 ml., it was found that the acetyl-isoniazid was in all cases completely eluted by 12 to 13 ml. of eluting fluid, the last 2 ml. of fluid coming through the column drug free.

In addition to the experiment shown in figure 3, separate solutions containing 20 γ of isoniazid and acetyl-isoniazid per ml. were placed on columns and were fractionally eluted in the same manner as described in the previous experiment. Even with these high drug concentrations, no contamination of the isoniazid fraction by acetyl-isoniazid or of the acetyl-isoniazid fraction

by isoniazid was observed. This indicated that no hydrolysis of acetyl-isoniazid occurred on the Dowex 50 × 8 resin as used in the test presented.

Recoveries of isoniazid, acetyl-isoniazid, pyridoxine hydrochloride, and PAS·Na are shown in table 1. Recovery of added isoniazid and acetyl-isoniazid from aqueous solutions and drug-free pooled serum was excellent, the quantity recovered being within plus-minus 5 per cent of the quantity added.

As may be seen from the table, apparently any portion of the isoniazid or acetyl-isoniazid which may have been bound to serum protein was removed upon passage through the cationic resin. Since pyridoxine and PAS are commonly used concomitantly with isoniazid, these were included. These two substances did not interfere. In addition, after the ingestion of 500 mg. of pyridoxine hydrochloride and 6 gm. of Ca·PAS by human volunteers on eight different trials, no interfering substances were detected in blood withdrawn two and six hours after administration

TABLE 1

RECOVERY OF ISONIAZID, ACETYL-ISONIAZID, PYRIDOXINE HYDROCHLORIDE, AND PAS·NA ADDED TO 3 MILLILITERS OF AQUEOUS SOLUTION, DRUG-FREE POOLED SERUM, AND URINE.

Drug	In γ per Ml.					
	Aqueous Solution		Serum		Urine	
	Amount Added	Amount Recovered	Amount Added	Amount Recovered	Amount Added	Amount Recovered
Isoniazid	1.00	1.00	1.00	1.05	1.00	1.00
	1.00	0.98	1.00	0.96	0.00	0.05
	1.00	1.00	1.00	1.00		
	1.00	1.00	0.80	0.80		
			0.50	0.52		
			0.20	0.20		
Acetyl-isoniazid	1.00	1.00	1.00	0.99	1.00	0.97
	1.00	1.02	1.00	1.00	0.00	0.07
	1.00	1.00	0.80	0.79		
	0.80	0.78	0.50	0.50		
	0.20	0.22	0.20	0.22		
	0.50	0.52				
Pyridoxine hydrochloride	50.0	0.0	50.0	0.0		
PAS·Na	50.0	0.0	50.0	0.0		

TABLE 2

BACKGROUND VALUES OBTAINED FROM 3 MILLILITERS OF DRUG-FREE SERUM CARRIED THROUGH THE ENTIRE PROCEDURE

(This serum was obtained from the same group of nontuberculous volunteers presented in table 3)

Subject	In γ per Ml.		Subject	In γ per Ml.	
	Background as Equivalent Concentration of Isoniazid	Background as Equivalent Concentration of Acetyl-Isoniazid		Background as Equivalent Concentration of Isoniazid	Background as Equivalent Concentration of Acetyl-Isoniazid
D. A.	0.07	0.04	M. L.	0.05	0.04
E. B.	0.05	0.05	Q. B.	0.06	0.05
H. O.		0.05	M. A.	0.06	0.04
S. M.	0.05	0.04	A. C.	0.06	0.03
E. N.	0.06	0.06	M. B.	0.05	0.02

of the drugs. Serum from these subjects processed through the outlined test gave no color at all; in fact all values were the same as those obtained on drug-free pooled serum.

Background values on drug-free serum from nontuberculous volunteers were run and are shown in table 2. This group of volunteers is the same group which is studied in table 3. As may be seen from table 2, the presence of substances in normal serum which might react with the reagents employed to give false readings for isoniazid and related compounds was insignificant, being so low that no blue color developed at all.

The drug concentrations of isoniazid and acetyl-isoniazid in serum of 11 nontuberculous volunteers, two hours and six hours after receiving 5 mg. or 2.5 mg. of isoniazid per kg. of body weight, are shown in table 3. With doses of 5 mg. of isoniazid per kg. of body weight, the concentration of free isoniazid varied from 4 to 55 per cent of the total isoniazid concentration (free isoniazid plus acetyl-isoniazid) present two hours after administration of the drug. With the smaller dose (2.5 mg. per kg.), 14 to 34 per cent of the initial isoniazid serum concentration at two hours was still present as free isoniazid four hours later.

It will be noted, however, that in some subjects (*D. A.*, *M. A.*, *M. B.*, *Q. B.*, *A. C.*) the concentration of isoniazid after six hours was roughly proportional to the initial dose. In others (*B. C.*, *E. N.*, *M. L.*, *H. O.*, *S. M.*) the free isoniazid concentration after six hours was independent of

TABLE 3

DRUG CONCENTRATIONS IN SERUM OF ELEVEN NONTUBERCULOUS VOLUNTEERS TWO AND SIX HOURS AFTER RECEIVING 5 MG. OF ISONIAZID PER KILOGRAM OF BODY WEIGHT, OR 2.5 MG. OF ISONIAZID PER KILOGRAM OF BODY WEIGHT

Subject	Time in Hours	5 Mg. per Kg.				2.5 Mg. per Kg.			
		In γ per Ml.			6-Hour Free / 2-Hour Total × 100	In γ per Ml.			6-Hour Free / 2-Hour Total × 100
		Free Isoniazid	Acetyl-Isoniazid	Total Isoniazid		Free Isoniazid	Acetyl-Isoniazid	Total Isoniazid	
					per cent				*per cent*
D. A.	2	5.10	1.33	6.43	55	4.68	0.75	5.43	34
	6	3.51	1.30	4.81		1.86	0.54	2.40	
M. A.	2	4.50	2.46	6.96	22	1.98	1.31	3.29	16
	6	1.53	1.16	2.69		0.53	1.04	1.57	
M. B.	2	5.64	3.99	9.63	16	2.30	1.95	4.25	14
	6	1.56	1.44	3.00		0.59	0.94	1.53	
Q. B.	2	6.60	3.76	10.36	10	2.40	2.04	4.44	17
	6	1.09	2.61	3.70		0.77	0.76	1.53	
A. C.	2	4.38	2.97	7.35	14	1.48	1.79	3.27	25
	6	1.00	2.12	3.12		0.83	0.91	1.74	
B. C.	2	1.36	5.22	6.58	4	1.48	1.90	3.38	16
	6	0.24	1.93	2.17		0.54	0.67	1.21	
E. N.	2	2.42	2.76	5.18	18	1.51	1.94	3.45	25
	6	0.91	2.30	3.21		0.85	0.76	1.61	
M. L.	2	3.35	3.39	6.74	13	2.79	2.04	4.83	21
	6	0.90	2.16	3.06		1.00	1.22	2.22	
H. O.	2	1.70	4.84	6.54	15	1.62	3.06	4.68	16
	6	0.96	2.34	3.30		0.75	0.87	1.62	
S. M.	2	4.00	3.43	7.43	13	1.80	1.87	3.67	20
	6	0.98	2.38	3.36		0.74	0.92	1.66	
E. B.	2					1.83	3.24	5.07	19
	6					0.98	1.82	2.80	

TABLE 4

DRUG CONCENTRATIONS IN SERUM OF SIX TUBERCULOUS PATIENTS ON A DAILY DRUG REGIMEN OF 5 MG. OF ISONIAZID PER KILOGRAM OF BODY WEIGHT IN TWO DIVIDED DOSES

(Samples were withdrawn two and six hours after administration of 2.5 mg. of isoniazid per kilogram of body weight)

	In γ per Ml.						6-Hour Free Isoniazid / 2-Hour Total Isoniazid × 100
	Free Isoniazid		Acetyl-Isoniazid		Total Isoniazid		
	2 Hours	6 Hours	2 Hours	6 Hours	2 Hours	6 Hours	
							per cent
1	0.99	0.87	2.48	1.56	3.39	2.43	26
2	1.77	0.55	0.98	1.00	2.75	1.55	20
3	1.50	0.91	1.20	1.38	2.70	2.29	34
4	1.15	0.96	1.98	0.91	3.13	1.87	31
5	1.76	0.39	1.03	0.61	2.79	1.00	14
6	2.30	0.58	1.93	0.83	4.23	1.41	14

the initial dose, indicating distinctly different rates of metabolism of isoniazid by different subjects.

Presented in table 4 are the serum drug concentrations obtained on a group of 6 tuberculous patients after receiving total daily dosages of 5 mg. per kg. of body weight in two divided amounts of 2.5 mg. per kg. each at twelve-hour intervals. Serum was withdrawn two and six hours after the patients received 2.5 mg. of isoniazid per kg. of body weight.

DISCUSSION

The data shown in figures 1 and 2 reflect the sensitive nature of the described test from 0.1 to 1 γ per milliliter of body fluid. In figure 1 may be seen the very narrow range of maximal absorption of the color developed when the color reaction was applied to isonicotinic acid. Not only the conformance of the color produced with the Bouger-Beer Law but also the reliability and

reproducibility of the column procedure in separating isoniazid and acetyl-isoniazid are illustrated in figure 2.

The elution bar graphs presented in figure 3 show the excellent separation of acetyl-isoniazid and free isoniazid and indicate the complete selectivity of the cationic column procedure.

Data from table 1 indicate the good recovery of added drugs (95 to 105 per cent of the isoniazid and acetyl-isoniazid) and show the non-interference of PAS and pyridoxine in the technique employed. Furthermore, as stated previously, administration of PAS and/or pyridoxine to human subjects does not produce metabolites which interfere with the test as presented. Duplicate determinations run on patients' serum have shown reproducibility of the same magnitude or better than the drug recovery figures presented in table 1. The duplicate determinations check on the average within plus-minus 1.5 per cent.

It is believed justifiable to consider that the values for acetyl-isoniazid are reasonably correct, as this metabolite is generally recognized as comprising the bulk of the metabolically altered isoniazid (4). However, it is acknowledged that, in measuring acetyl-isoniazid, small amounts of other isoniazid metabolites may be included, as isonicotinic acid and its conjugates are eluted from the column with this fraction. The possibility of interference from small amounts of other hydrolyzable pyridine-4-derivatives is also recognized. However, no interference from these sources was found in drug-free pooled serum, in serum from subjects who ingested PAS and/or pyridoxine, or in any of the individual serum samples shown in table 2.

Urine background values are negligible with the technique described. In any case, such large quantities of drug are usually present in the urine that, when the method is applied to this fluid, 0.5 ml. of a 1:100 dilution is usually required, and background will be even less than that noted in table 2.

The capacity of the cationic resin for isoniazid and acetyl-isoniazid was not completely studied. However, up to 1,000 γ of isoniazid and acetyl-isoniazid have been separated on 0.7 ml. of resin without loss. This indicated that one could, in practice, use body fluids containing large amounts of the drug and dilute the final evaporate with distilled water and then test various concentrations with the color reagent until a readable range was found.

It would be a good practice to carry a 0.5 γ per milliliter standard isoniazid and acetyl-isoniazid in drug-free pooled serum through the entire procedure to check on the color reaction, as well as to serve as a test of the optical density vs. concentration curve. This also, of course, would obviate any doubt of the constancy and stability of the reagents.

Further routine clinical application of this technique to urine and cerebrospinal fluid is in process, as well as additional studies of the effects of various other drugs, such as streptomycin and PAS, on the rate of isoniazid inactivation.

Summary

A sensitive method currently in use at this laboratory for the determination of small amounts of free isoniazid, acetyl-isoniazid, and total isoniazid is presented. The suitability of the test for routine application in clinical medicine is indicated. Although appearing unduly complicated, actually it will be found that, when all reagents have been prepared and glassware and equipment assembled, the test will proceed simply and rapidly, so that as many as twenty serum samples, including standards, may be completed in one working day without undue effort. Any clinical laboratory equipped with a spectrophotometer or colorimeter which has a range of 400 to 700 millimicrons may routinely utilize this test, as no special equipment is required other than that found in a routine hospital laboratory.

Sumario

La Determinación Cuantitativa de Pequeñas Cantidades de Isoniacida en los Humores Orgánicos, Usando una Resina Derivada del Intercambio Catiónico

Se presenta una técnica sensible empleada actualmente en el laboratorio de los AA. para la determinación de pequeñas cantidades de isoniacida libre, acetil-isoniacida e isoniacida total. Se indica la adaptabilidad de esta prueba para aplicación sistemática en la clínica médica. Aunque parece demasiado complicada, en la práctica se verá que, una vez preparados todos los reactivos y dispuestos la cristalería y el instrumental, la prueba marchará sencilla y rápidamente, de modo que permite disponer, sin mayor esfuerzo, hasta de veinte muestras de suero, incluso tipos de comparación, en un día de trabajo. Todo labora-

torio clínico provisto de un espectrofotómetro o colorímetro que tenga una escala de 400 a 700 milimicras puede utilizar sistemáticamente esta prueba, pues no requiere ningún instrumental especial, aparte del disponible en cualquier laboratorio de hospital.

Resume

Détermination quantitative de petites quantités d'isoniazide dans les milieux liquides de l'homme au moyen d'une résine échangeant les cations

Les auteurs présentent une méthode sensible courramment employée dans leur laboratoire pour determiner des petites quantités d'isoniazide libre, acétyl-isoniazide et l'isoniazide total. Ils soulignent le coté pratique de cet examen pour son application courante en clinique. Bien que cette méthode paraisse compliquée, on trouvera en pratique que, lorsque tous les réactifs et l'équipement ont été préparés, elle peut être suivie si simplement et si rapidement que l'on peut faire sans effort en un jour, jusqu'à 21 études sérologiques, y compris des examens standard. N'importe quel laboratoire équipé avec un spectrophotomètre ou un colorimètre ayant une étendue de 400 à 700 millimicrons peut utiliser cette méthode puisqu'elle ne nécessite pas un équipement spécial, autre que celui que possède un quelconque laboratoire d'hôpital.

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PAPER ELECTROPHORETIC SEPARATION AND ELUTION OF SOME ANTITUBERCULOUS DRUGS AND THEIR METABOLITES[1]

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INTRODUCTION

The reports of Hughes and her associates (1-3) on the specificity of metabolism of isoniazid aroused considerable clinical interest due to its obvious importance in tuberculosis therapy. Mandel and associates (4) and Morse and coworkers (5) observed that the administration of para-aminosalicylic acid (PAS) influenced the height and duration of the concentrations of free isoniazid in the blood.

Way and associates (6) studied the metabolism of PAS in man. They studied it in a group of five subjects and found that the amount of free PAS recovered in the urine ranged from 14 to 33 per cent of the total dosage administered. The two principal metabolites, acetyl *p*-aminosalicylic acid and *p*-aminosalicyluric acid, ranged from 28 to 63 and from 0 to 26 per cent, respectively, of the administered dosage. This compares with a range of 2.9 to 36.8 per cent for free isoniazid and 14.1 to 73.9 per cent for the acetyl metabolite as reported by Hughes (1).

Pyrazinamide has not been shown to increase the concentration of free isoniazid in the blood. Still, it is important to know the circulating level of pyrazinamide and pyrazinoic acid in studying this drug's therapeutic efficacy in tuberculosis because the acid shows none of the bacteriostatic properties of the amide. A method then is needed in the study of this drug, as well as in the study of isoniazid and PAS, to separate the drug from its metabolites in body fluids.

It seems strange that no attempt has been made, by American workers at least, to employ paper electrophoresis in studying the metabolism of these drugs in man. Cuthbertson and associates (7) studied the metabolic products of isoniazid in human urine, using both paper chromatography and paper electrophoresis. They separated isoniazid from its acetyl metabolite electrophoretically, using a phthalate buffer with a pH of 4, but did not investigate serum concentrations. A number of German articles (8-12) reported investigations of the paper electrophoretic mobility of isoniazid and PAS, but they seem to be principally concerned with the problem of serum albumin combination with these drugs. In 1957, Maher and colleagues (13) studied the serum protein paper electrophoretic patterns of tuberculous patients presenting therapeutic problems. This study confirmed the earlier reports of a marked decrease in albumin concentration with a concurrent increase in gamma globulin changes which reflect in a general way the progress of the disease process.

In this earlier study, the lowest albumin concentration encountered was 2.45 gm. in 100 ml. Assuming a molecular weight for albumin of 68,000, the concentration of free isoniazid in the blood of this patient two hours after administration being 6γ per ml. and the molecular weight of isoniazid being 137, the molar concentration of albumin (3.6×10^{-4}) was almost nine times greater than that of isoniazid (4.3×10^{-5}). Since this was the lowest concentration encountered, a low concentration of albumin can hardly be indicated as a problem in transport of isoniazid. Assuming albumin does indeed transport isoniazid, then it must be concluded that the albumin in these cases (13) was modified in a manner which rendered it incapable of transporting isoniazid but without alteration of its mobility characteristics; or, the isoniazid in these patients was altered in such a way as to render it unable to be transported by albumin. Indeed, Hever (11) observed that, when isoniazid is added to serum albumin *in vitro*, it is easily separable by paper electrophoresis.

Stahl (12) succeeded in separating isoniazid and PAS electrophoretically in paper using a Veronal buffer with a pH of 8.5 and ionic strength of 0.08. In this system, isoniazid moved only about 4 per cent of the distance traversed by PAS. However, protein-free solutions were employed and no attempt was made to separate the metabolites in this system. Considerable overlap

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[2] Present address: Box 312, Tripler U. S. Army Hospital, APO 438, San Francisco, California.

TABLE 1

DRUGS AND METABOLITES STUDIED AND THEIR ELECTROPHORETIC BEHAVIOR UNDER THE FIELD CONDITIONS SHOWN

Compound	Electrolyte	Ionic Strength	H	Current	Field Strength	Time	Direction of Migration	Average Migration Distance	Average Mobility ($\times 10^{-5}$)
				mg.		min.		mm.	
Isoniazid	0.022N H_3PO_4	0.088	2.4	4	7v cm^{-1}	120	Cathodic	91	1.78
	0.03M Veronal[a]	0.014	7.9	6	10v cm^{-1}	105	Cathodic	16	0.25
Acetyl isonicotinoyl hydrazine	0.022N H_3PO_4	0.088	2.4	4	7v cm^{-1}	120	Cathodic	63	1.25
	0.03M Veronal[a]	0.014	7.9	6	10v cm^{-1}	105	Cathodic	8	0.13
Isonicotinic acid	0.022N H_3PO_4	0.088	2.4	4	7v cm^{-1}	120	Cathodic	13	0.24
Sodium *p*-aminosalicylate (PAS)	0.022N H_3PO_4	0.088	2.4	4	7v cm^{-1}	120	Cathodic	9	0.18
	0.03M Veronal[a]	0.014	7.9	6	10v cm^{-1}	105	Anodic	72	1.63
Sodium *N*-acetyl-*p*-aminosalicylate	0.022N H_3PO_4	0.088	2.4	4	7v cm^{-1}	120	—	0	0
	0.03M Veronal[a]	0.014	7.9	6	10v cm^{-1}	105	Anodic	59	1.34
Sodium pascorbate	0.03M Veronal[a]	0.014	7.9	6	10v cm^{-1}	105	Anodic	72	1.63
Pyrazinamide	0.022N H_3PO_4	0.088	2.4	4	7v cm^{-1}	120	—	0	0
Pyrazinoic acid	0.022N H_3PO_4	0.088	2.4	4	7v cm^{-1}	120	Anodic	31	0.61

was observed when using this system with isoniazid and its principal metabolite, acetyl isoniazid.

The literature cited above encouraged the present writers to investigate the paper electrophoretic behavior of isoniazid, PAS, pyrazinamide, and their metabolites in both protein-free and in serum solutions in the hope of possibly exploiting this technique for studying the metabolism of these drugs in man. The purpose of this paper is to describe the results of the investigation and to discuss the feasibility of their employment in studying antituberculous drug and metabolite concentrations in the blood of man.

MATERIALS, EQUIPMENT, AND METHODS

The pooled human serum used in this study was serum nonreactive in tests for syphilis that was obtained from the serology laboratory of the hospital. The serum was sterilized by Seitz filtration. The isoniazid, pyrazinamide, PAS compounds, and hydrazones of isoniazid, as well as the acetyl-isoniazid and the pyrazinoic acid, were obtained from several sources.[3]

A "hanging strip" type of electrophoresis cell[4] was employed. The ultraviolet source used to locate the compounds on the paper strip after electrophoretic migration was a mercury vapor tube with a filter having a maximal transmission at 320 millimicrons.[5] To study the absorption spectra of the compounds after elution from the paper strip following electrophoretic migration a Beckman-Model D. U. spectrophotometer equipped with an ultraviolet accessory was used.

Relatively concentrated (0.25 to 1 per cent) aqueous solutions of the compounds studied were first prepared and these were diluted with either demineralized water or pooled, human serum to obtain the protein-free or serum solutions employed, respectively. These solutions were applied in an even band across the filter paper strips at the center in aliquots of 0.01 ml. per application by means of the mechanical applicator supplied with the electrophoresis assembly. Not more than two applications per strip were employed as it was observed that more resulted in a prohibitively

[3] The isoniazid was obtained from Hoffman-LaRoche, Inc., Nutley, New Jersey, and the pyrazinamide from Merck & Co., Rahway, New Jersey. The PAS compounds were very generously provided by Dr. Hellwig of Hellwig, Inc., Chicago, Illinois; the hydrazones of isoniazid were generously provided by Dr. Hettie Hughes of the Christ Hospital Institute of Medical Research, Cincinnati, Ohio. Dr. David J. Stanonis of this laboratory prepared the acetyl isoniazid and the pyrazinoic acid.

[4] Spinco Model R, Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

[5] Electric Light Assembly, MX-1291/PAQ, Sirchie Finger Print Laboratories, Philadelphia, Pennsylvania.

wide band due to chromatographic migration away from the origin. Hence, the amount of drug or metabolite actually applied to the paper was either 25 or 50γ.

Altogether 15 different buffer systems were investigated thoroughly. These included acetic acid: 5N, phosphoric acid: 0.02, 0.022, 0.05, 0.1, and 1.0 N, sodium phosphate plus phosphoric acid, sodium hydroxide: 0.005 and 0.01 N, and diethyl barbituric acid (Veronal®) plus its sodium salt. The pH of the systems studied ranged from 1.5 to 12.0, the ionic strength from 0.014 to 2.0, and the time from one-half to five and one-fourth hours. For the sake of brevity, only the results obtained with the two systems found to be best suited to the task have been shown here.

The separation of isoniazid, acetyl-isoniazid, isonicotinic acid, pyrazinamide, and pyrazinoic acid was effected using 0.022N, phosphoric acid with an ionic strength of 0.088 and a pH of 2.4 as the electrolyte. A constant current of 4 ma. was applied for two hours. The starting voltage was 200 to 220 while the final voltage was 170 to 180. The field strength thus approximated 7 volts per cm.

To separate PAS, acetyl-PAS, and sodium pascorbate (Na PAS-C), a solution of 3 gm. of sodium diethyl barbiturate and 3 gm. of diethyl barbituric acid in 1 liter of water was used as the electrolyte. This solution has an ionic strength of 0.014 and a pH of 7.9. A constant current of 6 ma. was applied for one and three-fourth hours. The starting voltage was 305 to 310 and the final voltage 295 to 300. The field strength was approximately 10 volts per cm.

In all the experiments reported, the electrophoresis cells were at room temperature, which varied from 22° to 24°C. When the runs were completed, the strips were removed from the cells and dried in a horizontal position for thirty

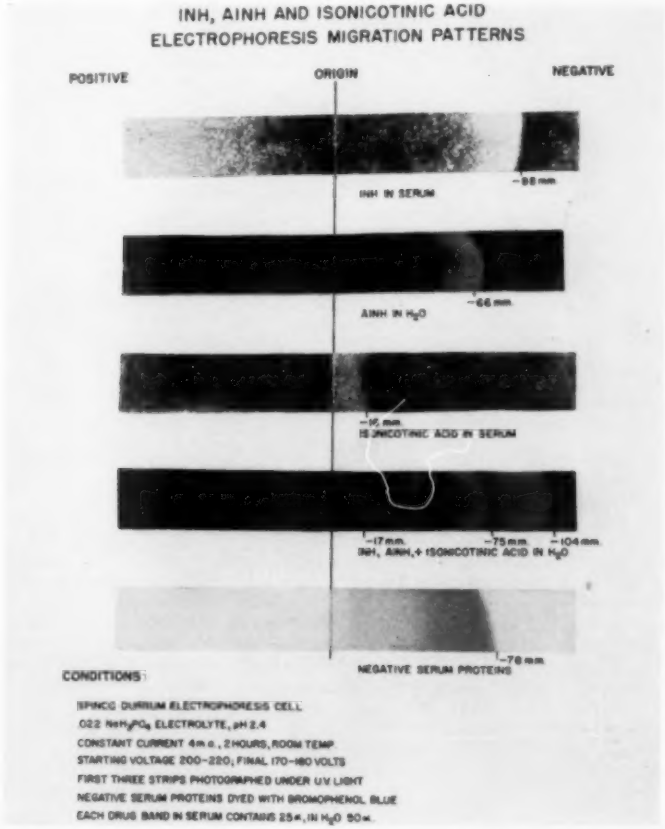
INH, AINH AND ISONICOTINIC ACID
ELECTROPHORESIS MIGRATION PATTERNS
POSITIVE
ORIGIN
NEGATIVE
INH IN SERUM
AINH IN H2O
ISONICOTINIC ACID IN SERUM
INH, AINH, + ISONICOTINIC ACID IN H2O
NEGATIVE SERUM PROTEINS
CONDITIONS:
SPINCO DURRUM ELECTROPHORESIS CELL
0.22 NaH2PO4 ELECTROLYTE, pH 2.4
CONSTANT CURRENT 4 m.a., 2 HOURS, ROOM TEMP.
STARTING VOLTAGE 200–220; FINAL 170–180 VOLTS
FIRST THREE STRIPS PHOTOGRAPHED UNDER U.V. LIGHT
NEGATIVE SERUM PROTEINS DYED WITH BROMOPHENOL BLUE
EACH DRUG BAND IN SERUM CONTAINS 25 γ, IN H2O 50 γ.

FIG. 1. Contact photographs showing the migration patterns obtained for isoniazid and two of its principal metabolites under the conditions shown. The pattern obtained with pooled human serum under the same conditions is shown for comparison. INH—isoniazid; AINH—acetyl-isoniazid.

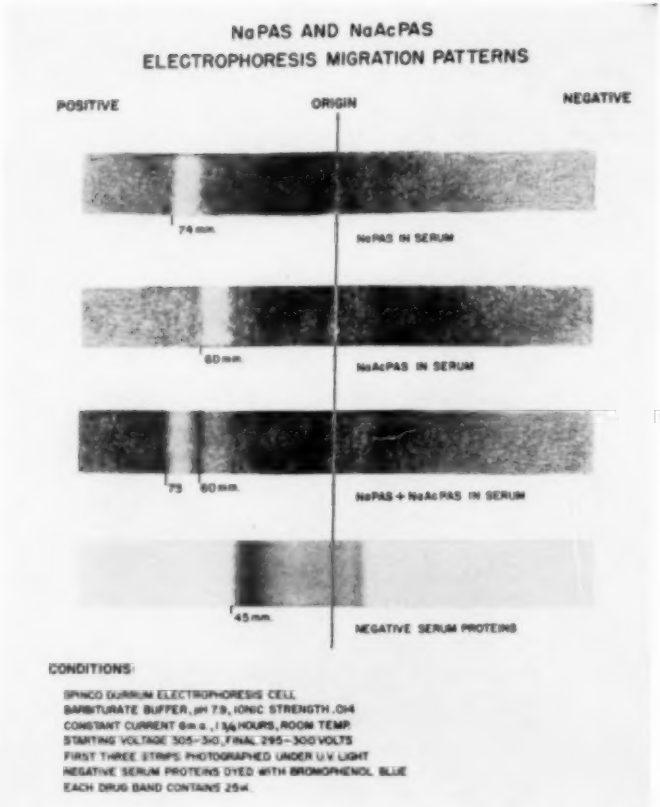

FIG. 2. Contact photographs showing the electrophoretic separation of NaPAS, NaAcPAS, and serum proteins, and the conditions employed.

minutes at 110°C. The origins were then marked with pencil and the strips viewed under ultraviolet radiation in a dark room. All of the PAS compounds fluoresced brilliantly under ultraviolet illumination and were readily visible. The other compounds absorbed some of the ultraviolet light and were thus visible as dark bands. The migration distance was measured from the origin to the leading edge of the band.

The photographs shown in figures 1, 2, and 3 were obtained by placing the strips in smooth contact with ultraviolet-sensitive paper and exposing them to the ultraviolet source described above. Two types of photo-sensitive paper were used. One[6] produced a light, speckled background, while the other[7] produced a heavy dark background.

[6] Kodagraph Contact Standard, Eastman Kodak, Inc., Rochester, New York.

[7] Apeco Green Seal, American Photocopy Co., Chicago, Illinois.

Elution of the compounds from the strip following electrophoretic separation was effected by cutting out the drug or metabolite band and transferring it to a test tube containing 3 ml. of 0.1 N hydrochloric acid. The paper was kept in contact with the acid for five minutes with occasional agitation, after which the acid was decanted and the procedure repeated using 2 ml. of acid. The acid eluate was then pooled and its absorbency at the wavelength of maximal absorption compared with that of a standard having the concentration equivalent to a quantitatively successful elution (100 per cent eluted). The reference solution was an eluate from a drug-free band of the same paper strip.

RESULTS

The conditions found optimal in this study for the electrophoretic separation in paper of isoniazid, PAS, pyrazinamide, and most of their

significant metabolites, are summarized in table 1. In the table the average migration distance of these compounds is also shown under the conditions described. This distance was measured from the origin or point of application to the leading edge of the band and is expressed in millimeters. The mobility represents the ratio of the distance traveled to the field strength.

Figures 1, 2, and 3 represent contact photographs of the results obtained in a few of the many experiments performed. The migration distances of the proteins of pooled human serum under the same conditions are shown for comparison. The proteins were stained with bromphenol blue and fixed with sodium acetate as described in the operating manual (14). Ordinary photographic techniques were employed in obtaining the photographs of these serum protein patterns.

The ultraviolet absorption spectra of the compounds studied after electrophoretic migration and elution from the filter paper strips are demonstrated in figure 4. The absorption spectra of the untreated compounds and the absorption spectra of a drug-free band of the same strip are shown for comparison.

The results of all the elution recovery experiments performed are presented in table 2, as well as the average recovery for each compound and the variance expressed as standard deviation.

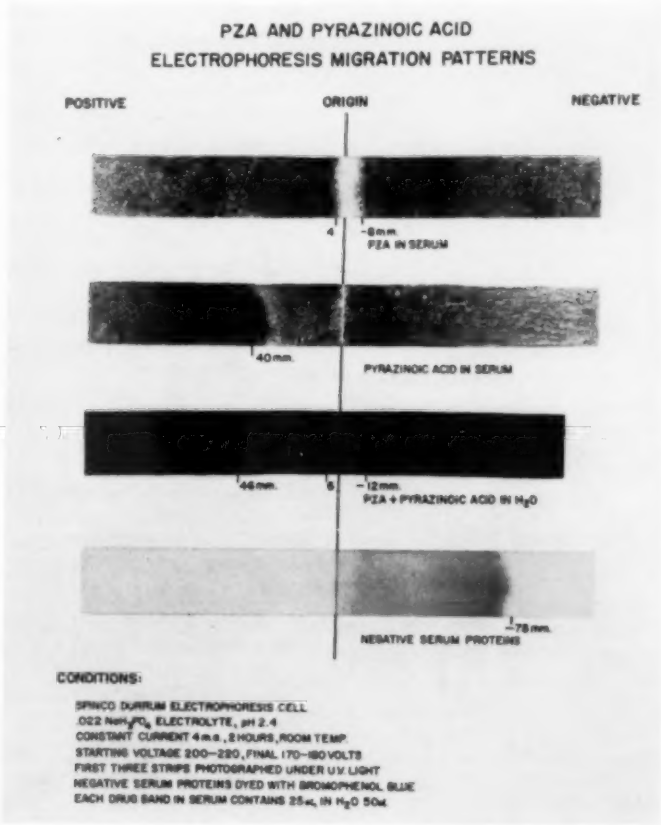

FIG. 3. Contact photographs showing the separation of pyrazinoic acid from pyrazinamide in serum under the conditions shown. The pattern of the serum proteins obtained under these conditions is presented for comparison. PZA—pyrazinamide.

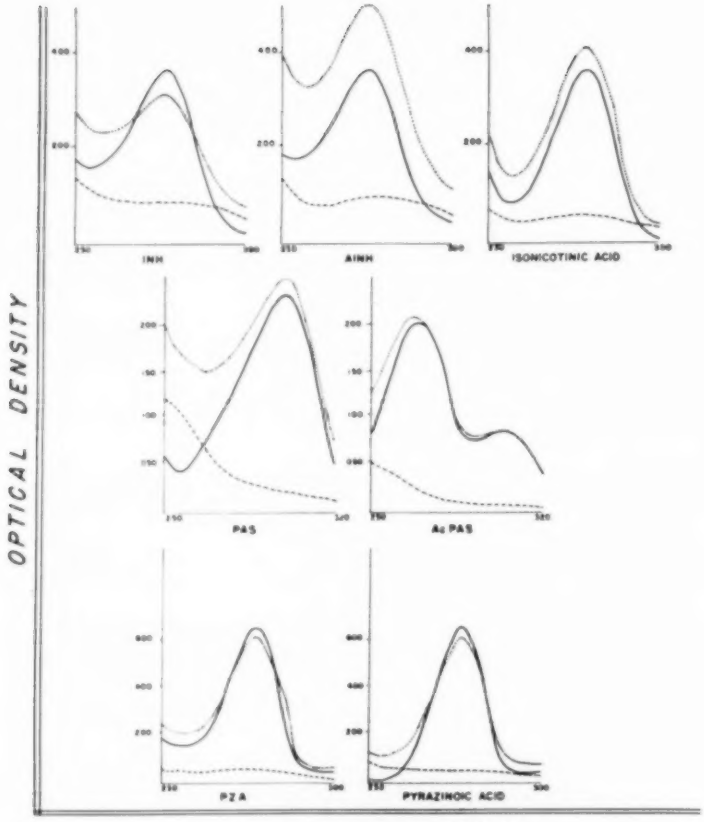

FIG. 4. The absorption spectra of the drugs and metabolites studied after elution from the filter paper strips (dotted curves). The spectra of the untreated compounds (solid curves) and of eluates from drug-free portions of the strip (dashed curves) are shown for comparison. The reference was distilled water. AINH—acetyl-isoniazid; PZA—pyrazinamide.

TABLE 2

SUMMARY AND INTERPRETATION OF ELUTION EXPERIMENT RESULTS

(In all experiments shown 50γ of each compound were applied to the paper prior to electrophoresis.)

Compound	From Serum Dilution						From Aqueous Solution					
	Per Cent Recovered				Average	Standard Deviation	Per Cent Recovered				Average	Standard Deviation
Isoniazid	64.2	46.8	58.9		56.6	7.3	62.1	66.6	56.2		61.6	4.3
Acetyl isonicotinoyl hydrazine	60.1	75.1	71.0	75.1	70.4	6.1	116.7 93.0	128.5 71.6	92.8	93.0	99.3	18.5
Isonicotinic acid	72.0	76.3	91.8		80.0	8.5	99.2	89.8	92.5	84.2	91.4	5.4
Pyrazinamide	70.2	85.6	84.6		80.1	7.0	84.5	89.6	91.6	99.0	91.2	5.2
Pyrazinoic acid	50.1	65.5	68.0		61.2	7.9	86.6	69.6	90.6	84.8	82.8	8.0
Sodium PAS	90.2	95.4	100.0		95.2	4.0	97.4	95.5	95.2	98.8	96.7	1.4
NaAcPAS	89.3	83.2	91.4		88.0	3.5	92.4	92.5	87.9	89.0	90.4	2.1

Discussion

From the data in table 1 it is apparent that good separation of these compounds electrophoretically in paper is feasible under the conditions shown. It is also apparent that isoniazid and its metabolites may be separated from pyrazinamide and its metabolites under the same field conditions, since the isoniazid compounds migrate toward the cathode, while pyrazinoic acid moves toward the anode and pyrazinamide remains at the origin. Para-aminosalicylic acid and its metabolites respond best to a pH of about 7.9 and an ionic strength of 0.014. Under these same conditions, isoniazid has only 25 per cent and acetyl isoniazid only 9.8 per cent, respectively, of the mobility of PAS and its acetyl metabolite. Sodium pascorbate, a combination of PAS with ascorbic acid, behaves exactly as does PAS under these conditions of electrophoresis.

In figure 1 is demonstrated the excellent separation of isoniazid from its principal metabolites, acetyl isoniazid and isonicotinic acid, obtainable using a phosphoric acid solution with a pH of 2.4 and an ionic strength of 0.088. The extreme instability of the hydrazone metabolites of isoniazid (alpha-ketoglutaric and pyruvic acid hydrazones) rendered impossible careful study of the electrophoretic behavior of these compounds in paper. The blood concentrations of these hydrazone metabolites must be investigated by other techniques. It can be seen from the serum protein pattern obtained under these same conditions that free isoniazid may be separated from the serum proteins under these conditions but not the two metabolites which migrate with the proteins. It is not suggested, however, that this coincidence of mobility implies any sort of chemical or physical bond between these metabolites and the serum proteins, since the metabolites' mobility in aqueous solution approximates their mobility in serum.

Also observed was a small but definite retarding effect on the mobility of isoniazid and its metabolites conferred by the serum proteins. This retarding effect becomes more marked as the bands move away from the origin. Thus, it was seen that free isoniazid in water moves 104 mm. and in serum 88 mm., while isonicotinic acid moves 17 mm. in water and 16 mm. in serum. This was interpreted as due simply to the physical interference of the proteins in the capillaries of the paper with the movement of the drugs through these same capillaries so that the longer the migration path of a compound the more marked was the protein-retarding effect.

An attempt to solve this problem of protein interference by heat denaturation of the proteins at the origin before applying the potential across the strip met with failure. It is still uncertain why, but the proteins heated to approximately 65°C. and, presumably thus denatured in the paper, had the same mobility characteristics as the untreated proteins under the same field conditions. Perhaps higher temperature and/or longer exposure will succeed in completely denaturing the proteins.

The photographs in figure 2 clearly demonstrate the feasibility of employing this technique for the separation of PAS from its principal metabolite, acetyl-PAS, and at the same time the separation of both of these compounds from the serum proteins. Although not shown in figure 2, the same protein-retarding effect described above was also observed in the investigation of the mobility of PAS and acetyl-PAS.

In figure 3 an almost ideal situation may be observed in that the serum proteins migrate toward the cathode while the principal metabolite pyrazinoic acid migrates toward the anode, the pyrazinamide remaining immobile at the origin. Thus, it appears theoretically possible to separate free isoniazid and pyrazinamide from serum proteins of a patient receiving these drugs in combination by using these experimental conditions.

The absorption spectra of figure 4 demonstrate that the electrophoretic treatment of these compounds in the manner described does not alter their absorption characteristics. It is reasonable to assume from this that the basic chemical structure of these compounds is unaltered by this treatment. The slight differences noted in these spectra are due to the absorption of the electrolyte eluted from the strip along with the compounds studied, so that, had the eluates from the drug-containing bands been referred to the eluate from a drug-free band of the same strip, the absorption spectra of the treated and untreated compounds should be perfectly congruous.

The data in table 2 reflect the weakest point of this technique, namely, the highly variable recovery on elution. There appears to be no logical explanation for this variability, but it is believed that it is somehow associated with the binding of the compounds to the paper and,

therefore, may be overcome by further investigation of eluting solvents other than hydrochloric acid and perhaps other types of filter paper.

Early in the study of this technique, it was noted that the sensitivity of 2.5γ per microliter was inadequate for investigating therapeutic concentrations in man which require a sensitivity some thousand fold greater. However, once the problem of controlling elution recoveries is overcome, it is probable that the sensitivity problem can be solved simply by using an increment method. That is, the therapeutic concentrations will be augmented in the specimen by the addition of a measured amount of the compound and the difference in optical density between incremented specimens and the increment solution itself will represent the concentrations actually present in the specimen.

Summary

The results of an investigation of the electrophoretic mobility in paper of three antituberculous chemotherapeutic agents and their principal metabolites are described. The mobilities of these compounds under the conditions found optimal in this study are reported. Photographs showing the separation obtainable in paper with the systems described are presented, and the retarding effect of the serum proteins on the electrophoretic mobility of the compounds is discussed. Variability of recovery of the compounds by elution from the filter paper after electrophoretic separation was the greatest handicap encountered.

Sumario

La Separación y Elución Electroforética en Papel de Algunas Drogas Antituberculosas y sus Metabolitos

Describense los resultados de una investigación de la movilidad electroforética en papel de tres agentes quimioterapéuticos antituberculosos y de sus principales metabolitos. Se presentan las movilidades de dichos compuestos en las condiciones que resultaron óptimas en este estudio. Ofrécense fotografías que muestran la separación obtenible en papel con el sistema descrito a la par que se describe el efecto retardador de las seroproteínas sobre la movilidad electroforética de los compuestos. El tropiezo mayor que se encontró consistió en la variabilidad observada en la recuperación de los compuestos por la elución desde el papel-filtro después de la separación electroforética.

Resume

Isolement et récupération par electrophorèse de quelques médicaments antituberculeux-leurs produits de metabolisme

Les auteurs décrivent les resultats de recherches sur l'electrophorèse de trois substances chimiques antituberculeuses. Les conditions optima de mobilité de ces produits sont présentées dans cette étude. Des documents photographiques montrent la séparation obtenue sur le papier dans cette méthode. Le role "retardant" des protéines sériques dans la mobilisation electrophorètique de ces produits est aussi discuté. La plus grande difficulté rencontrée fut la récuperation de valeur variable de ces produits par lavage du filtre, aprés la séparation electrophorètique.

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A COMPARATIVE STUDY OF NONPHOTOCHROMOGENIC MYCOBACTERIA AND MYCOBACTERIUM AVIUM[1]

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INTRODUCTION

Of the acid-fast bacilli which are distinct in colonial cellular and virulence characteristics from the usual tubercle bacilli, two groups have been distinguished: the photochromogenic and the nonphotochromogenic "Battey" type of mycobacteria (1, 2). These bacilli are considered to be presumptive pulmonary pathogens. The group of nonphotochromogens includes strains that usually develop slowly as smooth colonies with little or no pigmentation. Pigment occasionally present may be late to develop and is rarely of high intensity. These characteristics are common to a great number of acid-fast microorganisms whose distribution has not yet been determined. *Mycobacterium avium* has similar properties, and some nonphotochromogenic strains give evidence of relationship to this species. The objective of the present study was to determine if distinctive characteristics could be found for distinguishing nonphotochromogenic strains from *M. avium*.

MATERIALS AND METHODS

Strains of mycobacteria employed: Twenty-two strains of nonphotochromogenic Mycobacterium, 5 strains of *M. avium*, and one strain of *Mycobacterium* sp. *N. intracellularis* (Cuttino and McCabe, 1949 (3)) were utilized.[2]

Source of strains studied as nonphotochromogenic mycobacteria: From the National Tuberculosis Association-Veterans Administration Mycobacterial Culture Bank, Veterans Administration Hospital, Salt Lake City, Utah (Ernest H. Runyon):

Strain Number	Patient's Home	Specimens of Origin
P 2 (160)	Georgia	L
P 3 (170)	Georgia	S+, L
P 7 (357)	Texas	S+, L
P 17 (171C)	Georgia	S+, G, L
P 20 (223)	Nebraska	L
P 23 (264)	North Carolina	S+
P 25 (324)	New York	S+, L
225 A	Arizona	S+, G, L
248	California	L
277	Georgia	S+
470	Alabama	S+
477	Alabama	S+
487	Arizona	S+, G
509	Florida	S+, L
513	Florida	L
514	Florida	S+
520	Florida	S+, L
568	Texas	S+, L
584	Florida	S+, L
585	Florida	S+, L
586	Florida	S+, L
587	Florida	S+, L

Key: S = isolated from sputum
G = isolated from gastric juice
L = isolated from resected lung
+ = multiple specimens (sputum)
No tubercle bacilli isolated except possibly in *Case No. 587*.

Source of other strains:

M. avium	Kirschberg	Mayo Clinic	A. G. Karlson
M. avium	3589	Mayo Clinic	A. G. Karlson
M. avium	4133-26	Mayo Clinic	A. G. Karlson
M. avium	4109-2	Mayo Clinic	A. G. Karlson
M. avium	Sheard	Communicable Disease Center, Chamblee	G. P. Kubica
M. intracellularis		Communicable Disease Center, Chamblee	L. Ajello

Microscopic examination: The cultures were observed for acid-fastness and cellular morphology after a twelve-day incubation period at 37°C. on Löwenstein-Jensen medium. The Ziehl-Neelsen method of staining, as described by Gordon and Smith (4), was employed.

[1] From the Unidad de Patología, Escuela de Medicina, UNAM. Hospital General, México, 7 D. F., México.

[2] Cuttino and McCabe described this microorganism as *Nocardia intracellularis*. However, it is generally believed that this organism possesses well-defined characteristics of the genus Mycobacterium, as has been confirmed by the present writers. Hence it will be referred to in this paper as *Mycobacterium intracellularis*.

Growth in different culture media: The type of growth and pigment production in Proskauer and Beck liquid medium and in nutrient gelose with and without glycerol were recorded.

Acid from carbohydrates: The cultures were examined for acid production after seven and twenty-eight days' incubation at 37°C. on inorganic nitrogen agar, a modification of the medium of Ayers described by Gordon and Smith (4), with each carbohydrate autoclaved separately. Change of the indicator (bromo-cresol purple) to yellow indicates acid production.

Utilization of organic acids as carbon sources: Modifications of Koser's citrate agar (4) were made by combining 2 gm. of Na benzoate, Na citrate, Na succinate, Na pyruvate, or Na tartrate with a buffered saline solution, pH 6.8, agar 1.5 per cent, and phenol red as indicator. The alkaline color of the indicator after incubation at 37°C. demonstrates the utilization of an organic acid as a carbon source.

Decomposition of tyrosine: The cultures were streaked once across plates containing the following medium described by Gordon and Smith (4): peptone, 5 gm.; beef extract, 3 gm.; agar, 15 gm.; L-tyrosine, 5 gm.; distilled water, 1,000 ml.; pH 7.0. After an incubation period of fourteen to twenty-one days at 37°C., the plates were inspected for the disappearance of the tyrosine crystals underneath and around the growth.

Hydrolysis of casein: Skim milk or skim milk powder, suspended in the proper amount of water and an equal volume of 2 per cent aqueous agar, were autoclaved separately at 121°C. for twenty minutes, cooled to 47°C., mixed, and poured into sterile plates. Each culture was streaked on a plate, incubated at 37°C., and observed for clearing of the casein at seven and fourteen days (5).

Hydrolysis of gelatin: Duplicate plates of the above mentioned medium for the decomposition of tyrosine, in which 15 gm. of gelatin were substituted for the 5 gm. of L-tyrosine, were streaked once across with each culture. Hydrolysis was measured by Frazier's technique (6).

Hydrolysis of starch: In this case, 10 gm. of potato starch were substituted for the gelatin. Lugol reagent was utilized to detect the hydrolysis.

Peroxidase activity: Hydrogen peroxide, 30 per cent[3] was diluted 1:30 with distilled water to obtain fresh 1 per cent hydrogen peroxide. Sufficient catechol was dissolved in distilled water to obtain a 0.2 per cent solution. The working reagent, prepared just prior to use, consisted of a mixture of equal parts of these two solutions. Growth of the different strains on Löwenstein-Jensen medium was covered with the above reagent. The presence of peroxidase was indicated when the colonies turned brown to black within forty-five minutes (7).

[3] Manufactured by Merck & Co., as Superoxol.

Temperatures of growth: The cultures were inoculated on Löwenstein-Jensen tubes and incubated in water baths at 28°, 37°, 45°, and 52°C. during two weeks and examined for growth.

Catalase activity: A colorimetric procedure for measuring the amount of catalase activity described by Peizer and Widelock (8) was employed. The amount of peroxide remaining after incubation with suspensions of mycobacteria was detected by color formation: 0.0006 gm. of peroxide (0.2 ml. of 0.3 per cent peroxide) in 0.4 ml. of phosphate buffer pH 7 and 0.2 ml. of 6 per cent citric acid and 0.2 ml. of 1.1 per cent ammonium molybdate were mixed together, and the yellow color which appeared was taken as 100 per cent; other dilutions were made 0.0003 (50 per cent), 0.00015 (25 per cent), 0.00006 (10 per cent), and 0.000006 (0 per cent).

One loopful (3 mm. in diameter of 19-gauge wire) of young actively growing colonies of nonphotochromogenic and avian mycobacteria grossly suspended in 0.4 ml. of phosphate buffer, pH 7 in a test tube, and 0.2 ml. of 0.3 per cent peroxide were added. The culture-peroxide mixture was incubated in the refrigerator at 6°C. for thirty minutes. Then 0.2 ml. of 6 per cent citric acid and 0.2 ml. of 1.1 per cent ammonium molybdate were added in this order. Readings of results and comparisons with the color standard were made after the bacterial cells had settled to the bottom of each tube by centrifugation to 2,500 r.p.m. for five minutes.

Results

Microscopic examination: Under the conditions of this examination, the 22 strains received as nonphotochromogens and *M. intracellularis* showed cells usually smaller and of a different shape than typical tubercle bacilli, and often containing a single bead, rarely two. In the avian strains the presence of rods is more frequent than in nonphotochromogenic and *M. intracellularis* strains.

Growth in different culture media:

Proskauer and Beck liquid medium: After three to four weeks, very slight viscous to granular bottom growth without pigment was noted; turbidity and pellicle were not present.

Nutrient agar: After three to four weeks, dysgonic raised, regular, hemispherical creamy or white colonies were observed.

Glycerol agar: Eugonic development, raised, confluent creamy or white.

Löwenstein-Jensen medium: After three weeks, luxuriant, raised, confluent, creamy to

slight yellow growth with perfectly smooth surface was obtained.

These characteristics belong to all of the studied strains, nonphotochromogens, *M. intracellularis*, and *M. avium*.

Physiologic activity: The results of the physiologic tests applied to the 22 nonphotochromogenic mycobacteria, 5 *M. avium* strains, and that of *M. intracellularis* are presented in table 1.

The following characteristics were observed: Fourteen strains of nonphotochromogenic mycobacteria, one strain of *M. avium*, and the strain of *M. intracellularis* gave acid from glucose; 8 nonphotochromogenic and 4 avian strains failed to produce acid. Only 6 nonphotochromogenic strains were able to produce acid from mannose. All of the studied strains were capable of utilizing pyruvate and propionate. The other twenty-six tests were negative for all of the studied strains.

TABLE 1

OBSERVED CHARACTERISTICS OF NONPHOTOCHROMOGENIC AND AVIAN STRAINS OF MYCOBACTERIA

Acid from:	Nonphotochromogenic	*M. Avium*	*M. Intracellularis*
Glucose	±	∓	+
Galactose	−	−	−
Maltose	−	−	−
Lactose	−	−	−
Arabinose	−	−	−
Xylose	−	−	−
Mannitol	−	−	−
Sorbitol	−	−	−
Dulcitol	−	−	−
Raffinose	−	−	−
Mannose	∓	−	−
Rhamnose	−	−	−
Fructose	−	−	−
Inositol	−	−	−
Melibiose	−	−	−
Sucrose	−	−	−
Trehalose	−	−	−
Erythritol	−	−	−
Salicin	−	−	−
α-Methyl-D-glucoside	−	−	−
Utilization of:			
Benzoate	−	−	−
Citrate	−	−	−
Succinate	∓	−	−
Tartrate	−	−	−
Pyruvate	+	+	+
Propionate	+	+	+
Starch	−	−	−
Gelatin	−	−	−
Casein	−	−	−
Tyrosine	−	−	−

(+) Positive; (±) the majority gave positive results.

(−) Negative; (∓) the majority gave negative results.

TABLE 2

COMPARISON OF COLORIMETRIC CATALASE ACTIVITY TEST IN NONPHOTOCHROMOGENIC AND AVIAN MYCOBACTERIA

Number of Strains	Amount of Remaining Peroxide after Incubation with Suspensions of Mycobacteria				
	per cent				
	0.0	10	25	50	100
22 Nonphotochromogenic	8	4	6	2	0
5 *M. avium*	0	0	0	5	0
1 *M. intracellularis*	0	1	0	0	0

Catalase activity: Results of the catalase tests are recorded in table 2. The majority of the nonphotochromogens and *M. intracellularis* showed a higher catalase activity than *M. avium* strains; however, two nonphotochromogenic (Nos. P 25 and P 3) strains showed low activity similar to that of *M. avium*.

Peroxidase activity: In the present study, peroxidase activity was exhibited by the majority of the cultures: very slight activity was observed in some nonphotochromogenic strains (Nos. 277, 585, 513) and in the *M. avium* Sheard strain; absence of activity was observed in three nonphotochromogenic strains (Nos. 584, P 17, P 7).

Temperatures of growth: The 28 strains grew in temperatures from 28° to 37°C. The growth was luxuriant at 37°C. and scarce at 28°C. All avian strains but one (Sheard strain) grew well at 45°C., Sheard strain showed scanty growth; only 3 nonphotochromogenic (Nos. P 17, P25 and 509) grew at 45°C.; the other 19 nonphotochromogenic strains and *M. intracellularis* showed only scant growth at 45°C. At 52°C. not one of the studied strains was capable of growth.

DISCUSSION

The similarity of *M. avium* and the nonphotochromogens has been pointed out by Runyon (9) and Bogen (10). Crow and associates (2) and others have found the nonphotochromogens to be regularly avirulent for fowl, but avian and nonphotochromogenic strains have been reported to be very similar in other respects;

this was also found in the present study: colonial pattern, color, consistency, microscopic morphology, rate of growth, drug resistance, growth on different culture media, and scanty physiologic activity.

The difference in type of growth at 45°C. together with the low catalase activity of *M. avium* are not sufficient ground on which to make specific differentiation between this species and the nonphotochromogenic group. The existence of nonphotochromogenic strains with properties identical to those of *M. avium* makes this distinction impossible. These results indicate an important relationship between the nonphotochromogens and *M. avium*. *Mycobacterium intracellularis* appears to be a nonphotochromogenic Mycobacterium.

It is possible that virulence could be utilized as a criterion for separation of these acid-fast bacilli; however, experts in this field are not in agreement. Great variability in the virulence of *M. avium* for fowl is well known. The colonial type may be related to virulence (11). The possibility that *M. avium* could lose its virulence by prolonged existence in a heterologous host has also been indicated (12).

The above-mentioned data make the utilization of animal virulence unreliable for the characterization of these groups.

Knowledge of other properties, such as the utilization of different nitrogen sources, may help in identifying the nonphotochromogenic mycobacteria.

Summary

Twenty-two strains of nonphotochromogenic mycobacteria, 5 strains of *M. avium*, and one strain of *M. intracellularis* appeared to be very similar in colonial properties, microscopic morphology, physiologic activity, and peroxidase production. Only 3 of the nonphotochromogens grew at 45°C., while Sheard was the only strain of *M. avium* which failed to grow at this temperature. The majority of the nonphotochromogens showed higher catalase activity than *M. avium*. These slight differences, similar to the difference in virulence for fowl, are considered to be insufficient definitely to separate the nonphotochromogens from *M. avium*. In every test, *M. intracellularis* appeared to be identical with the nonphotochromogens.

Sumario

Estudio Comparativo entre las Micobacterias Nofotocromógenas y el M. Avium

En 22 estudios de micobacterias no fotocromógenas, 5 cepas del *M. avium* y de la *M. intracellularis* parecieron ser muy semejantes en sus propiedades coloniales, morfología microscópica, actividad fisiológica y producción de peroxidasa. Sólo 3 de las micobacterias nofotocromógenas proliferaron a 45°C., en tanto que la Sheard fué la única cepa del *M. avium* que no proliferó a dicha temperatura. La mayoría de las nofotocromógenas desplegó una actividad mayor de la catalasa que el *M. avium*. Se considera que estas leves diferencias, semejantes a las que existen para las aves de corral, son insuficientes para separar definitivamente las micobacterias nofotocromógenas del *M. avium*. En todas las pruebas, el *M. intracellularis* pareció ser idéntico a las micobacterias nofotocromógenas.

Resume

Etude comparée de bactéries nonphotochromogéniques et M. avium

Vingt-deux études de mycobactéries nonphotochromogéniques, 5 souches de *M. avium* et un de *M. intracellularis* semblant avoir les mêmes propriétés morphologiques, la même activité physiologique et la même production de peroxydase. Il n'y a que 3 nonphotochromogéniques bactéries qui poussèrent a 45°C, tandis que "Sheard" fut la seule sourche de *M. avium* qui ne se développa pas à cette température. La plupart des bactéries nonphotochromogéniques montrèrent une activité catalytique plus grande que *M. avium*. Ces légères différences, qui se retrouvèrent dans la différence de virulence vis à vis de l'animal, sont considérées comme insuffisantes pour séparer les bactéries nonphotochromogéniques de *M. avium*. Dans chaque étude, *M. intracellularis* s'est montré identique aux bactéries nonphotochromogéniques.

Acknowledgment

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STUDIES ON THE CATALASE ACTIVITY OF ACID-FAST BACILLI[1, 2]

I. An Attempt to Subgroup These Organisms on the Basis of Their Catalase Activities at Different Temperatures and pH

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INTRODUCTION

It is a well-known fact that acid-fast bacilli produce the enzyme catalase. Since the advent of the antimycobacterial drugs, however, it has been shown (1–4) that most strains of tubercle bacilli which become resistant to isoniazid will lose, or exhibit a lowering of, their catalase activity. This loss of catalase activity also has been correlated with attenuation in the pathogenicity of these microorganisms for the guinea pig (3, 4). The suggested correlation between catalase activity and pathogenicity of mycobacteria led Schweiger and associates (5) to determine the rate of catalase activity of various mycobacteria under certain specified experimental conditions. The results of these investigations indicated that virulent mycobacteria could be distinguished from avirulent and saprophytic strains by determining their rates of catalase activity at different temperatures and pH. It is the purpose of the present study to simplify the chemical procedure of Schweiger and to expand the investigation to include other varieties of acid-fast bacilli likely to be associated with human "tuberculosis" or with clinical materials isolated from suspected tuberculous patients.

MATERIALS AND METHODS

In the course of this investigation, 100 cultures of acid-fast bacilli were studied, including representatives of the following groups:

Human strains: Fifteen virulent human cultures were used: 3 of these were stock cultures (H37Rv, R_1, and dysgonic human); 10 were wild human strains; 2 were acquired from Dr. D. W. Smith, University of Wisconsin, Madison; and 3 came from Dr. A. A. Grover, The Christ Hospital, Cincinnati, Ohio. Four human strains each resistant to at least 10γ of isoniazid per ml. were obtained from Dr. A. A. Grover. The avirulent human strain, H37Ra, also was used.

Bovine strains: Five virulent bovine cultures were used: 2 of these (Ravenel and Copenhagen) were stock cultures; 2 were wild strains submitted to this laboratory for identification; and one was obtained from Dr. D. W. Smith, University of Wisconsin. A culture of BCG Birkhaug also was employed.

Avian strains: Of the 9 fowl-virulent avian cultures, 2 (Kirchberg and Sheard) were stock cultures, 4 came from the culture collection of Dr. D. W. Smith, and 3 were recent wild isolates. One avian strain (Jucho) was obtained from Dr. K. Takeya, Kyushu University, Fukuoka, Japan.

Saprophytic strains: Twelve stock strains of pedigreed saprophytes were used, 4 acquired from Dr. D. W. Smith, the remaining 8 being Communicable Disease Center stock cultures.

Unclassified acid-fast bacilli: Thirty-seven representatives of the unclassified acid-fast bacilli were obtained through the courtesy of Dr. Ernest H. Runyon, Veterans Administration Hospital, Salt Lake City, Utah. These included 12 *Group I* (photochromogen), 5 *Group II* (scotochromogen), 11 *Group III* (nonphotochromogen), and 9 *Group IV* (rapid growers, exclusive of *M. fortuitum*).

M. fortuitum: Nine cultures of *M. fortuitum* were used: 8 were obtained from the collection of Dr. D. W. Smith, and one from Dr. E. H. Runyon.

Members of the genus Nocardia: Three stock cultures of Nocardia were included in this study: *N. asteroides*, *N. madurae*, and *N. braziliensis*; all were obtained through the courtesy of Dr. Libero Ajello, Mycology Unit, Communicable Disease Center, Chamblee, Georgia.

Although the initial studies in this investigation were performed on cultures grown in Dubos-Middlebrook Tween[3]-albumin broth (6), it was soon found that much more definitive results could be obtained by using cultures grown on solid medium. For the period of this study, all cultures were grown and maintained on modified Löwenstein-Jensen coagulated egg medium (7). *Catalase tests* were performed in two ways. In one instance, 0.5 ml. of a 1:1 mixture of 10 per cent Tween 80[3] and 30 per cent hydrogen peroxide[4]

[1] From the Tuberculosis Unit, Communicable Disease Center, P. O. Box 185, Chamblee, Georgia.

[2] Presented in part before the Medical Session, as part of *Section 2B*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959.

[3] The polyoxyethylene derivative of sorbitan monooleate, obtained from Atlas Powder Company, Wilmington, Delaware.

[4] Superoxol, Merck and Company, Inc., Rahway, New Jersey.

was added directly to the culture slant held at room temperature. In determining the effect of temperature and pH on the catalase activity, several spadesful of growth were scraped from a culture slant and added to 0.5 ml. of the appropriate buffer solution; to this bacterial suspension 0.5 ml. of the Tween-peroxide mixture was then added. In all instances the catalase activities were recorded only as + or −, since the rapidity and extent of bubbling observed could be dependent upon several uncontrolled factors. Tubes which did not bubble immediately were observed for twenty minutes before discarding.

To determine the effect of temperature on the catalase activity, cultures were suspended in Sörensen phosphate buffer (8) of pH 7, and then placed in the appropriate water bath for twenty minutes prior to addition of the Tween-peroxide.

The effect of pH on catalase activity of the various acid-fast bacilli was studied, using the two extremes of pH 1 and pH 10. Culture growth was suspended in 1 ml. of the proper Clark and Lubs buffer (9) and allowed to stand at room temperature for twenty minutes before addition of the hydrogen peroxide. Studies are currently under way to determine the effect of combining pH changes with alterations in temperature.

Results

Initial studies were done on only a few representatives of the various groups of acid-fast bacilli previously described. These initial studies are recorded in table 1. From the data therein presented, it may be seen that the most clean-cut and differential results were obtained by studying catalase activities on the original slants (room temperature) and at 68°C. These two extremes of temperature permitted a differentiation of human and bovine varieties from all others in the table. On the basis of this preliminary study, the catalase activities of the 100 cultures described above were studied at the two temperature extremes indicated. The results of this large-scale study are recorded in table 2. From the data in this table, it is seen that the human and bovine strains, regardless of their virulence, can be readily distinguished from all other acid-fast bacilli listed, in that the former lose their catalase activity at 68°C. when suspended in pH 7 buffer, whereas other acid-fast bacilli are still able to break down hydrogen peroxide at this temperature.

The only instance wherein a discrepancy exists in this procedure is in the *Group III* or non-photochromogenic group of unclassified acid-fast bacilli. At 68°C., it will be noted that one of the 11 cultures tested showed no catalase activity; this variation from the norm seems to be the exception rather than the rule, for Jones reports (10) that of seventy-five recently isolated *Group III* unclassified acid-fast bacilli, all retained catalase activity at 68°C. It should be noted that these results are somewhat at variance with the data recorded in the abstracts of medical papers presented at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959 (11). The reason for this is that some of the catalase tests reported in the aforementioned abstract were performed on cultures grown in Tween-albumin broth. As has been mentioned earlier, data obtained from cultures grown in broth were neither as definitive nor as reproducible as those from cultures grown on solid medium.

TABLE 1

Preliminary Study: Effect of Variations in Temperature and pH on the Catalase Activity of Different Groups or Varieties of Acid-fast Bacilli

Strain*	pH†			Temperature‡			Original Slant at Room Temperature
	1	7	10	Room	56°	68°	
Human (8)	− to +	− to +	− to +	− to +	−	−	+
Bovine (3)	−	− to +	+	− to +	−	−	+
Avian (3)	−	− to +	+	− to +	− to +	+	+
Saprophyte (3)	− to +	+	+	+	+	+	+
Photochromogen (4)	+	+	+	+	+	+	+
Scotochromogen (1)	− to +	+	+	+	+	+	+
Nonphotochromogen (3)	−	+	+	+	− to +	− to +	+
Rapid grower (3)	− to +	+	+	+	+	+	+

* Numbers following strain indicate number of cultures used in this preliminary study.
† All pH variations performed at room temperature.
‡ All temperature studies performed in pH 7 buffer.

TABLE 2

CATALASE ACTIVITIES OF VARIOUS GROUPS OF ACID-FAST BACILLI AS OBSERVED AT ROOM TEMPERATURE AND 68°C.

Strain	Original Slant at Room Temperature*	pH 7 Buffer at 68°C.*
Human	+(19/19)	−(19/19)
Isoniazid-resistant humans	−(4/4)	−(4/4)
Bovine	+(6/6)	−(6/6)
Avian	+(10/10)	+(10/10)
Saprophyte	+(12/12)	+(12/12)
Photochromogen	+(12/12)	+(12/12)
Scotochromogen	+(5/5)	+(5/5)
Nonphotochromogen	+(11/11)	+(10/11)
Rapid grower	+(9/9)	+(9/9)
M. fortuitum	+(9/9)	+(9/9)
Nocardia species	+(3/3)	+(3/3)

* Fractional figure indicates total number of cultures reacting as indicated over total number of cultures tested.

DISCUSSION

In recent years, a number of *in vitro* tests have been proposed as a means of identifying the acid-fast bacilli (5, 12–20) either by group or virulence, or by both characteristics simultaneously. Most of these tests provide for a much more rapid subgrouping or virulence determination of cultures than is possible by the routine method of animal inoculation, a procedure which may take several months to complete. The catalase test herein described admittedly is not as quantitative as the procedure described by Schweiger; however, its speed of performance, simplicity, and reproducibility far outweigh what it lacks from the quantitative standpoint.

Perhaps a word should be said about the one nonphotochromogen culture which lost its catalase activity at 68°C. This was the culture #17 in Runyon's series, and a review of the literature reveals that this culture also has given erratic results when tested by other laboratories (12, 14). The possibility has been suggested (12) that this microorganism may not be a true nonphotochromogen but, rather, an as yet nebulous organism located taxonomically between the unclassified and avian group and other mycobacteria.

It must be recognized that these data were obtained primarily from laboratory stock strains. From the results obtained with the 15 wild strains included in the study, however, it is believed that this modified catalase test may prove to be a valuable tool for the rapid subgrouping of acid-fast bacilli. Then, too, this technique has been evaluated on recently isolated wild strains both in this laboratory and in the Georgia State Health Department (10). Of sixty-nine human strains, thirty-one photochromogens, and ninety-one Battey strains, a total of 191 cultures, all but three behaved as expected when subjected to this modified catalase test. Preliminary studies on other combinations of temperature and pH suggest that even further subdivision of the acid-fast bacilli may be possible through studies on catalase activity.

SUMMARY

The catalase activity of one hundred stock and recently isolated wild strains of acid-fast bacilli has been determined at room temperature and at 68°C. With the exception of four isoniazid-resistant human strains, all cultures of acid-fast bacilli exhibited positive catalase activity at room temperature.

It was found that the catalase enzyme of human and bovine strains (regardless of virulence) was inactivated by suspending the cultures in a phosphate buffer of pH 7 which was then held in a 68°C. water bath for twenty minutes. All other cultures tested retained their catalase activity under these conditions.

It is suggested that, pending further studies on wild strains, this modified catalase test may prove useful as a means of subgrouping the acid-fast bacilli.

SUMARIO

Estudios sobre la Actividad de la Catalasa de los Bacilos Acidorresistentes. I. Esfuerzo Encaminado a la Subdivisión de Estos Microbios a Base de Sus Actividades Catalásicas a Diversas Temperaturas y pH

Se determinó a la temperatura ambiente y a 68° C. la actividad catalásica de 100 cepas incultas, stock y recién aisladas, de bacilos ácidorresistentes. Exceptuadas 4 cepas humanas isoniacidorresistentes, todos los cultivos de bacilos ácidorresistentes revelaron actividad positiva de la catalasa a la temperatura ambiente.

Se observó que la enzima catalásica de las cepas humanas y bovinas (independientemente de su virulencia) se inactivaba al suspender los cultivos en un amortiguador fosfático de un pH de 7, que luego se mantenía a 68° C. en un baño-maría. Todos los demás cultivos comprobados re-

tuvieron su actividad catalásica en esas condiciones.

Se apunta que, hasta verificar estudios ulteriores con cepas incultas, esta prueba modificada de la catalasa puede resultar útil como medio de subdividir los bacilos ácidorresistentes.

Resume

Etude sur l'activité catalytique des bacilles acido-résistants. Essai de classification par sous-groupes de ces organismes en se basant sur leur activité catalytique à différentes températures et différents pH

On a déterminé l'activité catalytique d'un groupe de 100 souches et d'un groupe de souche récemment isolées, à température ambiante et à 68°C. A l'exception de 4 souches humaines résistantes à l'isoniazide, toutes les cultures de bacilles acido-résistants ont montré une activité catalytique positive à la température ambiante.

On a trouvé que l'enzyme catalase des souches humaines et bovines (sans tenir compte de leur virulence) était inactivé lorsque les cultures étaient placées dans une solution tamponnée avec des phosphates à un pH = 7 et étaient maintenues par la suite vingt minutes dans un bain d'eau à 68°C. Toutes les autres cultures mises à l'épreuve conservèrent leur activité catalytique dans les mêmes conditions.

Ce qui suggère, jusqu'à plus amples études sur des souches neuves, que l'on pourrait classifier par sous-groupes les bacilles acido-résistants en se basant sur la modification de leur activité catalytique.

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Case Reports

PULMONARY ASPERGILLOSIS[1]

ANGELO TOIGO[2]

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Introduction

Aspergilli are almost ubiquitous in nature and are a common contaminant in bacteriology laboratories. Although many species of the fungus have been distinguished, only a few are known to produce disease in humans. The species which causes human infection most often is *Aspergillus fumigatus* (1). Other species associated with the disease in humans are *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus flavus*, and *Aspergillus nidulans*. Although identification of species is difficult, identification of the genus is not. The appearance of a colony on Sabouraud agar is characteristic of all species of the genus; the colony resembles a field of ripening grain (1). Aspergillus infections are encountered, in the main, in people who have received antimicrobial or adrenal steroid hormone therapy for other diseases and those who are employed in farming or grain handling. It has also been described in people exposed to cotton dust (2, 3).

Despite the ubiquitous nature of aspergilli, pulmonary infection in humans is uncommon. Because bacteriologic specimens are easily contaminated by aspergilli, culture alone is not sufficient evidence of Aspergillus infection. In order to establish the presence of aspergilli as the cause of infection, the fungus must be cultured from sputum or other material and must also be demonstrated to be living in tissues (4).

The clinical manifestations of pulmonary Aspergillus infection vary widely, and no single pattern of disease is characteristic. The types of infection reported in the literature indicate the protean nature of the disease. These manifestations are acute lobar pneumonia (5), bilateral bronchopneumonia (6, 7), solitary lung abscess (3), intrabronchial mycetomas (8–11) with bronchiectasis, and disease patterns imitating pulmonary tuberculosis (12) or bronchogenic carcinoma (13). The case reported here is an example of a relatively rare disease that presented features not hitherto described.

Case Report

The patient was a 55-year-old white man admitted to the hospital on March 30, 1957, because of pain in the left side of his chest. He had been well until four weeks prior to admission when he began having pain in the lower left side of his chest. The pain was made worse by inspiration. He had a continuous cough and expectorated brown sputum for several days, and he was dyspneic. Two weeks prior to admission, the patient was seen by a physician who gave him one injection of penicillin as well as tablets to relieve the pain.

This patient had been employed as an agricultural worker for many years. Immediately prior to his illness, the man had been employed on a farm where he milked 47 cows daily but was not otherwise in contact with animals. As far as he knew, all of the cattle were healthy.

On admission to the hospital, examination revealed an acutely ill man with a temperature of 102°F. Dullness to percussion laterally was noted in the left thorax from the axilla downward, and flatness to percussion from the eighth rib downward posteriorly. Rales were heard throughout this area, and there were patchy areas of bronchial breathing. Whispered pectoriloquy was present over the area of flatness. The remainder of the examination was normal.

The initial hemogram showed a leukocyte count of 26,200 per cu. mm. with a differential count of 94 per cent neutrophils and 6 per cent lymphocytes. The erythrocyte count was 4,100,000 per cu. mm. The hemoglobin level was 12 gm. per 100 ml., and the hematocrit was 37 per cent. The urinalysis was normal, and the cardiolipin flocculation was negative. The erythrocyte sedimentation rate was 26 mm. per hour. *Neisseria catarrhalis* and gamma streptococci were recovered from a sputum culture. The fasting blood sugar was 109 mg. per 100 ml.; the nonprotein nitrogen was 28 mg. per 100 ml. The serum albumin was 2.1 and globulin, 3.8 gm. per 100 ml. Agglutinations to typhoid O, typhoid H, Proteus OX-19, Brucella, and paratyphoid A and B were normal. Stool examinations were normal, and the stool culture revealed no pathogens.

A chest roentgenogram obtained on admission (figure 1) showed a confluent nodular infiltrate

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FIG. 1. Roentgenogram on admission showing nodular and confluent nodular densities in the left lower lobe and small infiltrates in the right lower lobe adjacent to the hilum.

of the left lower lobe. This was interpreted as compatible with a lobar pneumonia.

Seven sputum specimens were examined for acid-fast culture and smear, and no organisms were recovered. Guinea pig inoculation was negative on two occasions. Sputum cultures for yeast and fungi on three occasions were sterile. An electrocardiogram was normal.

At the time of admission, the patient's temperature was 102.5°F. Blood cultures were sterile. The sputum culture was noncontributory. The patient was given 1,800,000 units of penicillin daily for one week. At the end of the week, since he was still febrile, penicillin was discontinued, and 3 gm. of chloramphenicol were given daily. After seventy-two hours of this therapy the patient's temperature became normal. Despite this clinical improvement, a chest roentgenogram eleven days after admission (figure 2) showed wide dissemination of infiltrations, especially to the upper lobes of both lungs. The leukocytosis persisted, and the hemoglobin level gradually dropped from 14.5 to 11 gm. per 100 ml. The anemia was unexplained, and the patient was given 1,000 ml. of fresh whole blood.

The rales in the left lower lobe persisted, and more were heard in the left and right upper lobes. A roentgenogram on April 24, 1957, twenty-five days after admission to the hospital (figure 3), showed clearing of the infiltrates in the left lower lobe, but many cavities were observed in both upper lobes. Bronchoscopy revealed diffuse injection of the bronchial mucosa. Scalene lymph node and femoral lymph node biopsy examinations were described as showing chronic lymphadenitis. On April 24, 1959, two days before the patient died, a chest roentgenogram revealed further resolution of the infiltrates in the left lower lobe. Abscesses persisted and new nodular infiltrates were seen in both upper lobes (figure 4).

On the same day, the patient began to expectorate what appeared to be small pieces of lung tissue and fresh blood. Copious, bloody expectoration occurred with signs of peripheral collapse. The patient died twenty-seven days after admission.

Autopsy findings: The significant findings were in the lungs. The left lower lobe and both upper lobes were solid. There were numerous small nodular densities below the apical portions of both upper lobes and in the left lower lobe. A section through the apex of each lung showed a diffuse bronchopneumonia with innumerable small abscesses containing yellow mucoid material and necrotic lung tissue.

Microscopic examination of the lungs demonstrated numerous thick-walled abscess cavities. There was a heavy growth of fungus in the walls of these cavities (figures 5 and 6). *Aspergillus niger* grew in pure culture from this material.

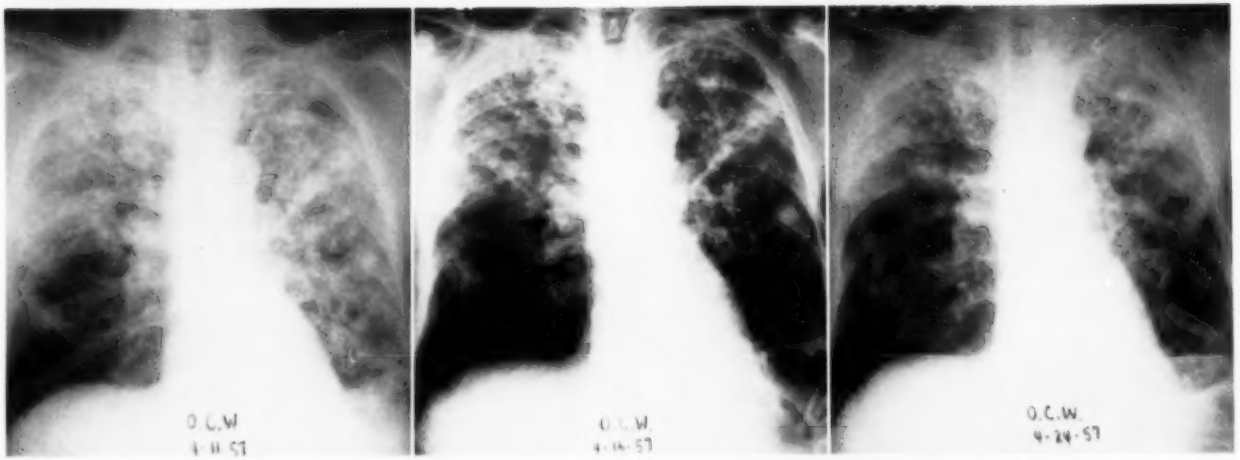

FIG. 2 (Left). Roentgenogram obtained eleven days later. Nodular and confluent nodular densities in all lobes of both lungs.

FIG. 3 (Center). Roentgenogram obtained seventeen days after admission. Nodular and confluent nodular infiltration of upper lobes of both lungs, resolution of the infiltrative process in the left lower lobe, and multiple abscess formation.

FIG. 4 (Right). Roentgenogram obtained twenty-four days after admission. Nodular and confluent nodular densities in both upper lobes and the right lower lobe. Small abscesses are seen in both upper lobes.

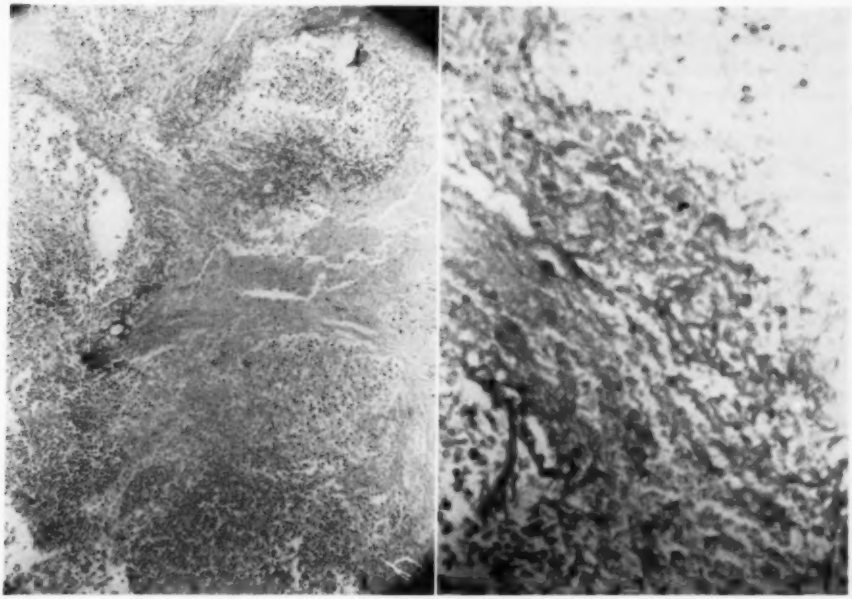

FIG. 5 (Left). Section of lung (× 440). The lower portion of the photomicrograph shows round cell infiltrate and fibrin. Two other abscesses are seen with mycelia growing in the abscess walls.
FIG. 6 (Right). Abscess wall (× 660) showing mycelia in greater detail.

No single bleeding point could be found to account for the massive terminal hemorrhage. There was no evidence of Aspergillus infection in other organs, and the remainder of the postmortem examination was unremarkable.

DISCUSSION

Although abscesses have been described as pulmonary lesions caused by Aspergillus, they are usually single or, if multiple, involve only one lobe. The most striking features of this case were the rapid spread of the disease and the formation of multiple abscesses in both upper lobes. Furthermore, the disease spread during a period of clinical well-being while there were no symptoms and no clinical signs of disease. The patient's death was due, not to systemic dissemination of infection, but presumably to the extension of an abscess into a major branch of the pulmonary artery. Indirect evidence for this was the absence of infection of other organs by Aspergillus and the relative normality of the remainder of the organs.

In view of widespread disease, it was puzzling that Aspergillus was not recovered from the sputum despite repeated attempts to culture it. At autopsy the material lying within the abscess cavities was extremely thick and tightly adherent to the walls of the cavities. One can surmise that perhaps for these reasons it could not be expelled.

Aspergillus infections occur as primary disease; infections secondary to other chronic illnesses, such as pneumoconiosis (14) or tuberculosis (15); and as infection following prolonged therapy with antimicrobial drugs or adrenal steroid hormones. When it occurs during the course of another infection, such as tuberculosis, it is probably a secondary invader and is not primarily responsible for the clinical illness. When Aspergillus infections follow prolonged use of antimicrobials or adrenal cortical steroid hormones, there have already been marked changes in the bacterial flora of the body and in the responses to infection which permit the establishment of aspergillosis and other fungal infections as almost an agonal event.

Experimental Aspergillus infection has been produced in animals by Sidransky and Friedman (16). They showed that the number of animals

infected by air-borne Aspergillus spores could be increased moderately by giving the animals either antimicrobials or adrenal cortical hormones, and the number increased markedly by giving them both.

The clinical analogue to this situation (development of Aspergillus infection following long-term antimicrobial and adrenal cortical steroid hormone therapy) has been described by several writers (17–20). In the cases described, both pulmonary and disseminated aspergillosis followed treatment with these drugs.

The case presented here is probably one of primary aspergillosis because the patient had received only a single injection of penicillin prior to admission, had a well-established pulmonary infection when he was admitted, had not received steroid hormone therapy during the course of this illness, and because no other disease was revealed at post-mortem examination.

Summary

A case of pulmonary aspergillosis with several unusual features is described. The disease disseminated rapidly throughout both lungs while the patient felt and appeared well. There were multiple abscesses in both lungs. A sudden, lethal pulmonary hemorrhage occurred, but the source of this could not be demonstrated at autopsy.

Sumario

Aspergilosis Pulmonar

Se describe un caso de aspergilosis pulmonar, dotado de varias caracteristicas extrañas. La enfermedad se diseminó rápidamente a través de ambos pulmones mientras que el enfermo se sentia bien y parecia hallarse así. Había abscesos múltiples en ambos pulmones. Hubo una súbita hemorragia pulmonar letal, pero no pudo descubrirse en la autopsia la causa de la misma.

Resume

Aspergillose pulmonaire

L'auteur décrit un cas d'aspergillose pulmonaire avec plusieurs traits inhabituels. Cette maladie se propagea rapidement dans les deux poumons tandis que le malade se sentait et apparaissait être en bonne santé. Il y eut de multiples abcès dans les deux poumons. Il se produisit une hémorragie foudroyante mortelle dont l'origine ne put être découverte à l'autopsie.

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SUBCOSTAL EXTRAPERIOSTEAL PLOMBAGE FOR MASSIVE TUBERCULOUS HEMOPTYSIS[1,2]

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Introduction

The safety and efficacy of resection therapy in the elective surgical management of patients with pulmonary tuberculosis has been amply demonstrated, and its value as an emergency measure for the patient with massive tuberculous hemoptysis has also been confirmed (1–5). The purpose of this paper is not to deprecate the role of excisional surgery in instances of massive hemorrhage due to tuberculosis but to suggest that, in specific circumstances, subcostal extraperiosteal plombage continues to warrant careful consideration in the management of this grave complication. During the ten-year period from 1949 through 1958, 4 patients with life-threatening tuberculous hemoptysis were treated by extraperiosteal plombage on the thoracic surgery services of the Boston City Hospital. The course of events in these patients forms the basis of this report. In the same interval on this hospital service, there were no resections for life-threatening tuberculous hemoptysis.

Case Reports

Case 1. A 60-year-old Negro was admitted to the hospital in October, 1947, with bilateral far advanced pulmonary tuberculosis. Sputum was positive for acid-fast bacilli on microscopy. A chest roentgenogram demonstrated a mottled, soft infiltration throughout the right lung and involving the left midlung field as well; there was a 3 cm. cavity in the right apex. Laryngeal tuberculosis constituted an added problem.

Therapy with strict bed rest for a period of nine months effected moderate improvement, but the patient remained infectious, and a 1 cm. cavity persisted in the right upper lobe. Gross hemoptysis began in August, 1948, with daily amounts of blood progressing from 90 to 450 ml. in a five-day period. Right phrenemphraxis was then performed, following which the amount of bleeding was greatly reduced although not eliminated. After one month the phrenic crush was augmented with pneumoperitoneum. Blood-streaking occurred intermittently thereafter but did not constitute a serious problem until February, 1949, when the patient suddenly expectorated 500 ml. of bright red blood. Large pneumoperitoneum refills were only temporarily successful in controlling the bleeding, and two days later hemoptysis amounting to 300 ml. occurred. This was followed by a day of lessened bleeding, after which bleeding recurred massively, the patient producing 1,300 ml. of blood in an eight-hour period. Large pneumoperitoneum was without benefit. The patient's general status began to deteriorate rapidly and he experienced increasing difficulty in maintaining a patent airway because of the hemorrhage. A chest roentgenogram demonstrated persistence of the right upper lobe cavity. Bronchoscopy indicated that the source of bleeding was the right upper lobe.

It was believed that the continuing hemorrhage was life-threatening and that surgical intervention offered the only hope of arresting it. However, because of poor general condition, sputum positive for *M. tuberculosis*, and impaired pulmonary function, the risks of resection were considered prohibitive. Consequently, under endotracheal general anesthesia with nitrous oxide–oxygen–ether, emergency seven-rib right subcostal extraperiosteal plombage was performed, using a total of 24 hollow one-inch Lucite spheres. Transfusion of 1,500 ml. of whole blood was administered during the operation. Recovery from emergency surgery was satisfactory and the patient was free of hemoptysis for eight days. Bleeding then recurred in amounts which ranged from simple streaking to 60 ml. daily, but after three weeks no further bleeding was noted.

Seven months after the operation the patient had gained 50 pounds, his general condition was greatly improved, the roentgenographic appearance was stable, and there had been no hemoptysis for six months. He produced an estimated 15 ml. of mucoid sputum daily and his sputum and gastric washings had been negative for *M. tuberculosis* since one month after operation. However, in October, 1949, guinea pig inoculation of the gastric aspirate was reported to be positive for *M. tuberculosis*, although culture of the same specimen had been negative. In view of the patient's age and satisfactory home situation, he was discharged on October 28, 1949. Frequent subsequent gastric aspirates were consistently negative for tuberculosis and the

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patient experienced no further pulmonary bleeding. In 1957 he succumbed to a cerebral hemorrhage at the age of seventy, his tuberculosis having been arrested for almost ten years.

Comment: This patient's plombage was the first to be performed on the thoracic surgery service of the Sanatorium Division, Boston City Hospital, and was considered a desperate measure at the time. However, the immediate and long-term response to operation was highly gratifying, since hemorrhage was arrested and disease controlled. With the improved present-day chemotherapy, it is probable that the initial results of medical management would have been better and that plombage, if indicated, would not have been deferred until it became imperative.

Case 2. The patient was a 47-year-old white male who entered the sanatorium in March, 1948, because of far advanced bilateral pulmonary tuberculosis. Sputum was positive for acid-fast bacilli on direct microscopy. A chest roentgenogram showed extensive infiltrative disease bilaterally with cavitation in both infraclavicular areas. Initial therapy included bed rest and a total of 25 gm. of streptomycin administered in the amount of 1 gm. biweekly. Progress was slow, however, and reversal of infectiousness was not achieved until seventeen months after admission. There was clinical evidence of brief improvement at this juncture and the patient gained 7 pounds in weight.

In November, 1949, brisk hemoptysis occurred but ceased promptly after pneumoperitoneum had been instituted. The patient's appetite became poor and his weight was not maintained. The sputum again became positive for *M. tuberculosis*. Clinical deterioration was accompanied by worsening of the roentgenographic findings which, in February, 1950, showed enlargement of the bilateral apical cavities, particularly on the left. Bronchoscopy on March 1, 1950, showed chronic endobronchitis but no ulceration or stenosis. A bilateral permanent collapse program was considered but was rejected because of the patient's poor general condition and greatly impaired ventilatory function. Serious hemoptysis recurred abruptly on March 27, 1950, and the patient daily produced amounts of blood ranging from 120 to 180 ml. Pneumoperitoneum was ineffective in controlling the hemorrhage. The patient became fatigued and experienced difficulty in maintaining a patent airway by coughing. It was evident after ten days that the bleeding would end fatally if allowed to persist but that open thoracotomy and resection would probably result in death. It was therefore decided to attempt subcostal plombage.

One liter of whole blood was administered by transfusion in preparation for the operation. Bronchoscopy was performed on April 6, 1950, under general anesthesia, preliminary to operation, and showed brisk hemorrhage on the left. Although the lobar bronchial orifices could not be delineated because of the bleeding, the side of origin was readily established. A seven-rib left subcostal extraperiosteal plombage was then accomplished, using 31 2-cm. Lucite spheres. The patient received 2,000 ml. of whole blood by transfusion during the procedure. His status at the completion of surgery was precarious, but it gradually improved. Brisk hemorrhage ceased shortly after operation, and slight streaking persisted for only two days thereafter. Despite early encouraging signs, the patient began to deteriorate one week after operation, with dyspnea, marked weakness, and low-grade fever. His course was downhill and he died on the sixteenth postoperative day.

Comment: Although emergency plombage was effective in controlling hemorrhage in this debilitated patient, pulmonary function was so poor that his death was essentially hypoxic. Resection would undoubtedly have had as unfortunate an outcome.

Case 3. A 42-year-old white female was a chronic alcoholic with serologic evidence of syphilis. Because of tuberculosis of the right upper lobe, she had been initially hospitalized in 1951 for a fourteen-month period, leaving the sanatorium against advice. She was readmitted in March, 1953, but again was self-discharged one month later. A similar sequence was followed from September to November, 1954, and from June, 1955, to June, 1956; on all occasions the discharge was voluntary and against medical advice. Episodes of relatively brisk hemoptysis had occurred in October and November, 1955, and in January and February, 1956. Chest roentgenograms continued to demonstrate a large cavity in the right upper lung field. The sputum was consistently positive for acid-fast bacilli on direct smear. Therapy included streptomycin, isoniazid, PAS, and pneumoperitoneum in various combinations and for various intervals. Because of the patient's recalcitrance, it was impossible to maintain a rational and effective course of treatment.

The patient was readmitted for the last time in October, 1956, extremely ill, emaciated (weight, 88 pounds), and febrile. Sputum was positive for acid-fast bacilli on direct smear. A chest roentgenogram showed extensive exudative and productive lesions throughout the right lung with a 6 by 2.5 cm. cavity above the first rib, a 1.0 cm. cavity at the level of the third right posterior rib, and a 2.5 cm. cavity in the third right anterior interspace; dense infiltration and "honey-combing" were noted in the left upper lung field. Treatment with streptomycin, PAS, and isoniazid was reinstituted, but the patient made slight progress. Diarrhea, fever, hemoptysis, epigastric pain, and an anxiety state were complicating problems.

After four months, however, the patient had

gained 10 pounds in weight and her sputum had become negative for *M. tuberculosis* by both microscopy and culture. On April 16, 1957, brisk hemoptysis recurred and persisted. The patient coughed frequently and raised bright red blood with each expectoration. The bleeding reached alarming proportions, and the patient began to experience difficulty in maintaining a patent airway. It was evident that the hemorrhage would not cease spontaneously and would terminate fatally if allowed to continue. Because of bilateral disease, uncertain drug coverage (despite sputum noninfectiousness), and a virtually moribund condition, emergency pulmonary resection was not considered feasible. A chest roentgenogram showed persistence of the large right upper lobe cavity with little change in the disease elsewhere. The hematocrit on the evening of April 23, 1957, was 34 per cent. Hemorrhage accelerated and the hematocrit fell to 31 per cent on the following morning despite transfusion of 1,000 ml. of whole blood. Bronchoscopy showed the source of bleeding to be the right upper lobe orifice. It was decided to proceed promptly with a seven-rib subcostal extraperiosteal plombage on the right. This was done under general anesthesia using a Carlens tube to provide bronchial compartmentation and thus prevent contralateral spillage of blood, which by now was vigorously issuing from the right upper lobe.

During the operation, which was satisfactorily tolerated, the patient received 1,500 ml. of whole blood by transfusion. The general condition continued poor, however. Hemoptysis persisted after operation but at a reduced rate. The patient was tachypneic (40–45 per minute), and roentgenograms on the first postoperative day showed patchy atelectasis. Tracheostomy was performed to facilitate endotracheal suctioning and improve ventilation. The hematocrit on the second postoperative day was 43 per cent. The amount of expectorated blood continued to abate and by the fifth day after plombage it had ceased entirely. The patient's general status showed gradual improvement and the respiratory rate approached normal. A course of slow but satisfactory progress was interrupted on the twenty-fifth day after plombage by an episode of hematochezia. This was managed by multiple transfusions of whole blood. A large hematemesis occurred on the twenty-seventh postoperative day and, despite blood repletion, the patient rapidly declined and died twenty-eight days after plombage. Autopsy showed an acute gastric ulcer which had bled massively.

Comment: This woman was a classic example of the recalcitrant tuberculous patient. Symptomatic improvement because of drug therapy was invariably followed by self-release, so that an effective regimen could not be maintained. Emergency plombage was undertaken because of grave hemoptysis and successfully eliminated this hazard. The use of a Carlens tube for intubation was highly satisfactory in preventing contralateral spillage of blood during the operation. Tracheostomy constituted a helpful measure in the early postoperative period. The lethal acute gastric ulcer was undoubtedly precipitated by the stress of surgery.

Case 4. The patient, a 63-year-old white man, was a chronic alcoholic with known hepatic cirrhosis. The presence of esophageal varices had been confirmed esophagoscopically in the past, and the patient had experienced at least one episode of hematemesis. A fibrotic lesion had been known to be present in the apex of the left lung for several years. Sputum had been positive for acid-fast bacilli at the time the lesion was discovered, but there had been no recent bacteriologic studies. Therapy had included streptomycin and PAS for an undetermined period in the past. On October 9, 1957, blood-spitting recurred and on the following day the patient was admitted to the Boston City Hospital.

Physical examination showed stigmata of cirrhosis, including ascites. The patient was producing bright red blood by mouth. Initially it was thought that there was recurrence of bleeding from the varices. A portable chest roentgenogram demonstrated bilateral apical fibrocaseous tuberculosis of uncertain activity, with a poorly delineated cavity on the left. Bronchoscopy on October 10, 1957, indicated that the hemorrhage was pulmonary in origin and that its specific source was the left upper lobe bronchus. Therapy with streptomycin, PAS, isoniazid, bedrest, and low-sodium diet was instituted. Sputum smears for acid-fast bacilli were unsatisfactory because of the abundant admixture of blood. The hemoptysis gradually worsened and the hematocrit fell from 44 to 34 per cent. The patient began to experience difficulty in maintaining a patent airway because of the bleeding. It was believed that the continuing hemorrhage was life-threatening in itself and might precipitate hepatic coma. Because of lack of coverage with antituberculous drugs, severe alcoholism, liver damage, uncertain bacteriologic status, and poor general condition, resection was thought to entail an unwarranted risk. Nevertheless, control of the bleeding seemed imperative. Consequently, it was elected to perform subcostal plombage.

On October 17, 1957, under general anesthesia, a left seven-rib subcostal extraperiosteal plombage was performed,[3] 35 large (1.5 inch) and 5 small (1 inch) spheres being inserted. The anesthetic mixture was administered through a carefully positioned Carlens tube for bronchial compartmentation because of the gross bleeding. The operative procedure was well tolerated and the patient recovered satisfactorily. Hemoptysis

[3] By Dr. Irving M. Madoff.

ceased within twenty-four hours of operation. On judicious medical management, which included continuation of antituberculous drugs, the patient made gradual progress. Ascites regressed and laboratory studies of liver function demonstrated improvement. Two sputum smears were negative for acid-fast bacilli.

The patient declined portacaval anastomosis and was discharged on January 29, 1958. Despite an episode of acute alcoholic intoxication shortly after discharge, he was in generally good health one year later. He continued to receive long-term antituberculous drug therapy, and the cirrhosis was satisfactorily managed by medical means.

Comment: The tuberculous etiology of the hemorrhage in this patient seemed a reasonable assumption in view of the past history and presence of bilateral apical disease with cavitation on the left. Because of proved varices, bronchoscopy was required to differentiate hemoptysis from hematemesis. Among other compelling reasons, the lack of antituberculous drug coverage suggested that plombage might be preferable to resection. Contralateral spillage of blood was prevented intra-operatively by means of a Carlens tube. Hemoptysis ceased after plombage and the long-term results to date have been equally satisfactory.

Discussion

Massive bleeding, such as that from the gastrointestinal tract, is notoriously difficult to define and quantitate satisfactorily, and most studies are concerned with the hemodynamic effect of the acute blood loss. With hemoptysis the problem of "massive" bleeding becomes even more indefinite since there is the added and paramount factor of obstructive asphyxia. Because functioning lung parenchyma may already be reduced by extensive tuberculosis, the hazard of asphyxia is correspondingly greater. Exsanguination per se is an unlikely occurrence with pulmonary bleeding, although the circulatory effects of the blood loss should always be considered. The characterization of hemoptysis as "massive" must be related to its duration and to the general state of the patient, as well as to the absolute amount of bleeding, since a patient with slow but continuous hemorrhage may become fatigued from coughing and find it impossible to maintain an airway. Thus, a large amount of brisk bleeding in a vigorous individual is not comparable with a smaller amount of continuous hemorrhage in an elderly debilitated patient, in whom such bleeding might well be considered "massive." For the purpose of this paper, bleeding has been considered "massive" when it was thought to be life-threatening in itself if permitted to continue or to recur. A similar definition has been proposed by Ehrenhaft and Taber (6).

The rationale of using "coagulants" such as vitamin K, carbazochrome (Adrenosem®), toluidine blue, and congo red in serious pulmonary bleeding is poorly established, but they have been employed empirically on the services in this hospital. Pneumoperitoneum and phrenemphraxis are more logical procedures and have occasionally been found effective. Temporary tracheostomy has proved to be of considerable value in permitting ready aspiration of blood clot in patients who otherwise have difficulty in maintaining an airway; it may also improve ventilation by reducing the anatomic dead space.

Bronchoscopic localization of the source of bleeding is considered mandatory when surgical intervention is contemplated. It may be performed with increased risk under general anesthesia immediately prior to operation, but is done preferably with topical anesthesia at an earlier time. Physical findings, the subjective impression of the patient, and roentgenography may suggest localization but are not wholly reliable. In general, it is the writers' opinion that bronchoscopy should be undertaken promptly in *all* patients with significant hemorrhage in order to document the site of origin.

Unless there is acceptable roentgenographic evidence of cavitation in an upper lung field, the decision to perform subcostal plombage for control of serious pulmonary hemorrhage rests on tenuous grounds. Such cavitation was present in all of the patients in the present series, although in one (*Case 4*) the cavity was ill defined. It should be mentioned that the necessity of using a portable X-ray machine in critically ill and tachypneic patients may result in technically imperfect films which fail to demonstrate ideally delineated cavitation.

Antimicrobial therapy and bacteriologic status, closely related factors, are most important in deciding whether to perform resection or plombage in the patient with acute massive bleeding. Such hemorrhage is more likely to occur when antimicrobial therapy has been sporadic or poorly controlled and, under these circumstances, plombage offers a greater margin of safety than

does resection. Similarly, the majority of seriously bleeding patients whose drug therapy has been erratic are demonstrably or presumably infectious at the time surgery becomes imperative. In these patients the potential morbidity and mortality of resection is considerably greater than that of plombage.

The pulmonary function of all patients in the present series was considered marginal, and this was a major factor in electing to perform plombage rather than resection. Ventilatory studies made during a bleeding episode are not valid, however, and the estimate of pulmonary function has been based on clinical evaluation except in instances in which such studies had antedated the hemorrhage.

As indicated above, subcostal plombage for massive hemoptysis presents several advantages not shared by resection. Since open thoracotomy is not performed, the likelihood of pleural complications is negligible. The potential loss of ventilatory function is less than it is with resection. Subcostal plombage also may favorably affect disease in areas other than the source of hemorrhage, such as the superior segment of a lower lobe. In general, plombage as an operative procedure is better tolerated than resection, and this difference is magnified in the poor-risk patient. Admitted disadvantages of plombage include the fact that a collapse operation is never as definitive as excisional surgery. It is usually possible to occlude the appropriate bronchus at an early stage during resection, a technical advantage not shared by plombage. In addition, properly executed resection results in immediate control of hemorrhage while, after plombage, hemostasis may be relatively gradual. Although uncommon, massive bleeding from an arteriobronchial fistula (2) would probably not be amenable to plombage.

Summary

Four patients with massive tuberculous hemoptysis have been treated by extraperiosteal subcostal plombage. In all patients the bleeding was satisfactorily controlled. Resection admittedly remains the ideal and definitive mode of treatment for massive tuberculous hemoptysis but, in such selected cases as those presented, the use of plombage may be preferable. All operations have been performed under endotracheal general anesthesia, and on two occasions a Carlens tube was employed to prevent contralateral spillage of blood. Bronchoscopic localization of the source of hemorrhage is mandatory before proceeding with surgical intervention. Roentgenographic demonstration of cavitary disease is important if plombage is contemplated. It is suggested that extraperiosteal subcostal plombage may be the operation of choice for control of massive tuberculous hemoptysis in the poor-risk patient with impaired pulmonary function, uncertain antimicrobial drug coverage, and infectious sputum.

Sumario

El Plumbaje Extraperióstico Subcostal para la Hemoptisis Tuberculosa Masiva

Cuatro enfermos con hemoptisis tuberculosa masiva han sido tratados con el plumbaje subcostal perióstico. En todos se cohibió satisfactoriamente la hemorragia. La resección continúa siendo reconocidamente la forma ideal y definitiva de tratamiento para la hemoptisis tuberculosa masiva, pero en casos escogidos como los presentados, el uso del plumbaje puede resultar preferible. Todas las operaciones se ejecutaron con anestesia general endotraqueal y en dos ocasiones se empleó una sonda de Carlens para impedir la extravasación contralateral de sangre. Es imperativa la localización broncoscópica del punto de origen de la hemorragia antes de pasar a la intervención cruenta. Es importante la observación radiográfica de enfermedad cavitaria si se piensa en el plumbaje. Se señala que el plumbaje subcostal extraperióstico tal vez sea la operación de elección para el dominio de la hemoptisis tuberculosa masiva en el enfermo que constituye un mal riesgo y tiene afectada la función pulmonar y si hay incertidumbre en cuanto al uso de drogas antimicrobianas y el esputo es infeccioso.

Resume

"Plombage" sous-costal et extrapériosté dans des cas d'hémoptysies massives d'origine tuberculeuse

Quatre malades présentant une hémoptysie massive d'origine tuberculeuse ont été traités par "plombage" extrapériosté et sous-costal. Chez tous ces malades l'hémorragie fut contrôlée d'une façon satisfaisante. De l'avis de tous, la résection reste le moyen définitif et idéal de traiter une hémoptysie massive tuberculeuse, mais dans des cas comme ceux-qui sont présentés içi, l'emploi du "plombage" peut être préferable. Toutes les opérations ont été effectuées sous anesthésie générale endotrachéale et à deux occasions, on employa un tube de Carlens pour éviter que l'autre poumon soit envahi par du sang. Il faut obliga-

toirement localiser par bronchoscopie la source de l'hémorragie avant l'intervention chirurgicale. L'évidence radiographique d'une forme avec caverne est importante si on envisage un "plombage." Les auteurs recommandent le "plombage" sous-costal et extrapériosté comme l'opération de choix pour contrôler les hémoptysies tuberculeuses massives chez les malades en mauvais condition, avec une fonction respiratoire altérée, protégés d'une façon incertaine par des antibiotiques et ayant une expectoration positive.

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Notes

TRANSFORMATION OF ISONIAZID AND STREPTOMYCIN RESISTANCE IN MYCOBACTERIUM AVIUM BY THE DESOXYRIBONUCLEATE DERIVED FROM ISONIAZID- AND STREPTOMYCIN-DOUBLE-RESISTANT CULTURES

Since Avery and co-workers[1] reported that desoxyribonucleic acids (DNA) extracted from an encapsulated "smooth" strain of pneumococcus would transform unencapsulated cells producing "rough" colony types into encapsulated cells, a number of studies on the bacterial transformation of certain properties including drug resistance have been reported [2-6] (references have been reviewed by Hotchkiss).[5, 6]

It was a most striking fact that, as reported by Hotchkiss,[3-6] there were a number of parallels between the manner in which bacterial mutant characters were acquired spontaneously and the manner in which they were transferred by DNA. Previously, the present writers have reported that various degrees of isoniazid resistance ranging from 3 to 50 γ of isoniazid in a strain (Jucho) of *Mycobacterium avium* are determined by the same genotype.[7] This result has suggested that the isoniazid resistance of the organism is produced by only a single mutational step, that is, by a single gene mutation. If this is true, it might be expected that DNA preparations extracted from any isoniazid-resistant mutants of *M. avium* would transform susceptible cells at a single step into isoniazid-resistant cells. This should be the case whether the isoniazid-resistant mutants utilized as sources of DNA preparations had been obtained by a single-step or by multiple-step selection. It is, therefore, the purpose of the present paper to test such a possibility.

A streptomycin- and isoniazid-double-resistant strain isolated from *Mycobacterium avium*, strain Jucho, has been employed. The strain was isolated by multiple-step selection, first with streptomycin and thereafter with isoniazid. The selection was made by cultivating the strain in modified Sauton medium containing a graded series of drug dilutions, followed by subculturing of surviving organisms obtained at the level of partial inhibition. With this procedure, streptomycin resistance reached as high a level as more than 10,000 γ of streptomycin after multiple-step selection and, on the other hand, isoniazid resistance reached as high a degree as 20 to 40 γ of isoniazid resistance in a single step, but the selection was continued repeatedly. Therefore, it has been believed that the strain, which is resistant to more than 10,000 γ of streptomycin and, simultaneously, also to more than 50 γ of isoniazid, has been obtained as the result of multiple cumulative mutations to streptomycin resistance and the single high step to isoniazid resistance.[8]

The media used were modified Sauton and Sauton agar of a composition previously described.[7] The methods of determining the number of survivors and of calculating in the total viable population the ratio of mutants resistant to isoniazid or streptomycin were also the same as described previously.[7]

The DNA was extracted by a modified Chargaff-Saidel method.[9] Seven-day-old cultures of the streptomycin- and isoniazid-double-resistant mutant strain of *M. avium* in Sauton medium were washed with distilled water and treated with a tenfold volume of acetone. The cells treated were extracted with ether at 0°C. for twenty-four hours and ground with glass powder at 0°C. for sixty minutes. The paste obtained was extracted with a twenty fold volume of borate buffer, pH 7.6, containing 0.01 M sodium citrate at 0°C., for twenty-four hours, and centrifuged at 3,000 r.p.m. for thirty minutes. The supernatant fluid was again centrifuged at 12,000 r.p.m. for twenty minutes. The supernatant fluid thus obtained was added to one volume of 1 per cent sodium desoxycholate, allowed to stand, and was stirred several times at 0°C. for twenty-four hours. Protein impurity was repeatedly removed according to the method of Sevag, and the fluid obtained after the treatment was added to two volumes of alcohol and centrifuged. The precipitate was dissolved in distilled

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[2] Alexander, H. E., and Leidy, G.: J. Exper. Med., 1953, *97*, 17.

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[9] Sasaki, T.: Kekkaku, 1957, *32*, 505.

water and dialyzed at 0°C. for twenty-four hours against running water. The DNA fluid thus obtained was thereafter again dialyzed at 0°C. for twenty-four hours against distilled water and lyophilized. The yield of DNA was approximately 600 mg. from 170 gm. of acetone-treated powder of cells. The amount of DNA was measured by the diphenylamine method.

The DNA preparation was also extracted from the parent drug-susceptible strain by the method described above.

The desoxyribonucleic acid preparation extracted from the streptomycin- and isoniazid-double-resistant strain was dissolved with a small volume of distilled water and added to alcohol. The precipitate was washed with ether and dried. The DNA preparation thus sterilized was dissolved with Sauton medium to give a final concentration of approximately 1 mg. of DNA per ml. of medium. The Sauton medium, 50 ml. in an Erlenmeyer flask, added to the resistant DNA, was inoculated with 2 mg. of a culture of the par-

TABLE 1

POPULATION STRUCTURE OF CULTURES GROWN ON SAUTON MEDIUM ADDED TO DESOXYRIBONUCLEIC ACID (DNA) EXTRACTED FROM THE STREPTOMYCIN- AND ISONIAZID-DOUBLE-RESISTANT STRAIN (RESISTANT DNA), AND TO DNA EXTRACTED FROM THE PARENT-SUSCEPTIBLE STRAIN (SUSCEPTIBLE DNA) OF *M. AVIUM*, STRAIN JUCHO

Isoniazid	Culture Added to Susceptible DNA		Culture Added to Resistant DNA	
	Number of Survivors*	Survival Ratio	Number of Survivors*	Survival Ratio
(γ *per ml.*)				
0	35.8 ± 9.21	1.00	52.4 ± 25.9	1.00
1	26.6 ± 12.7	0.742	42.8 ± 6.18	0.818
2	23.2 ± 8.35	0.649	14.2 ± 3.04	0.271
3	6.3 ± 3.41	0.176	2.6 ± 1.22	4.95×10^{-2}
4	0.0	$<5.58 \times 10^{-3}$	2.4 ± 1.73	4.57×10^{-2}
5	0.0	$<5.58 \times 10^{-3}$	2.2 ± 1.32	4.19×10^{-2}
10	0.0	$<5.58 \times 10^{-3}$	1.5 ± 1.23	2.86×10^{-2}
10	27.4 ± 7.14†	7.65×10^{-4}	238.6 ± 33.2‡	4.55×10^{-2}

Population structure was determined by using modified Sauton agar medium containing 3 per cent agar.

* (Mean of five replicates) ± (standard deviation).

† Determined by inoculating 10^8-fold size of inoculum per plate.

‡ Determined by inoculating 10^2-fold size of inoculum per plate.

TABLE 2

POPULATION STRUCTURE OF CULTURES GROWN ON SAUTON MEDIUM ADDED TO DESOXYRIBONUCLEIC ACID (DNA) EXTRACTED FROM THE STREPTOMYCIN- AND ISONIAZID-DOUBLE-RESISTANT STRAIN (RESISTANT DNA) AND TO DNA EXTRACTED FROM THE PARENT-SUSCEPTIBLE STRAIN (SUSCEPTIBLE DNA) OF *M. AVIUM*, STRAIN JUCHO

Streptomycin	Culture Added to Susceptible DNA		Culture Added to Resistant DNA	
	Number of Survivors*	Survival Ratio	Number of Survivors*	Survival Ratio
(γ *per ml.*)				
0.0	35.8 ± 9.21	1.00	52.4 ± 25.9	1.00
0.1	30.4 ± 10.3	0.849	46.0 ± 6.12	0.875
0.2	20.8 ± 9.87	0.581	35.4 ± 23.8	0.674
0.3	1.2	3.35×10^{-2}	33.0 ± 8.50	0.628
0.4	0.0	$<5.58 \times 10^{-3}$	23.0 ± 20.1	0.437
0.5	0.0	$<5.58 \times 10^{-3}$	12.6 ± 2.02	0.240
1.0	0.0	$<5.58 \times 10^{-3}$	0.0	$<3.82 \times 10^{-3}$
10	0.0†	$<5.58 \times 10^{-8}$	0.0†	$<3.82 \times 10^{-8}$

Population structure was determined on modified Sauton agar medium containing 3 per cent agar.

* (Mean of five replicates) ± (standard deviation).

† Determined by inoculating 10^8-fold size of inoculum per plate.

ent-susceptible strain of *M. avium* and incubated at 37°C. After five days of incubation, the ratios of cells resistant to various concentrations of isoniazid and streptomycin were determined. The control experiment consisted of similar procedures except that the DNA preparation extracted from the parent-susceptible strain was used.

The results obtained are shown in tables 1 and 2. As shown in table 1, only a few survivors capable of growing on medium containing 3 γ of isoniazid and no survivors capable of growing on medium containing more than 4 γ of isoniazid were found among several hundred viable cells of the control culture. Survivors capable of growing on medium containing 10 γ of isoniazid were found at a rate of 7.65×10^{-8} in the control culture. On the other hand, such survivors were found at a rate of 4.55×10^{-2}, that is, at a rate approximately six hundred fold higher in the culture containing the DNA extracted from the resistant cultures than in the control culture. Furthermore, survivors capable of growing at more than 4 γ of isoniazid have been found at almost similar rates. This indicates that the mutational step which produced such survivors has never occurred in quantitative mutational steps, but only in a single step. This does not appear to reflect the existence of different DNA particles determining different degrees of isoniazid resistance, but the existence of DNA particles determining more than 10 γ of isoniazid resistance in all-or-none fashion. Therefore, it has appeared that isoniazid resistance in *M. avium* is produced by single gene mutation.

Ratios of cells in the total viable population resistant to various concentrations of streptomycin are shown in table 2. Most of the individual cells grown in the control culture added to the DNA extracted from the susceptible culture were resistant to 0.3 γ of streptomycin but were not resistant to 0.4 γ of streptomycin. On the other hand, those grown in the culture added to the DNA extracted from the resistant culture were resistant to 0.5 γ of streptomycin. The rate of appearance of mutants resistant to as much as 10 γ of streptomycin did not appear to be different in both cultures, as observed in the present study.

Subsequently, the results obtained appear to indicate that transformation of streptomycin resistance occurs in quantitative mutational steps and to reflect the history of isolation of the resistant strain used for the present experiment. The results obtained also agree with those obtained previously by Katsunuma and Nakasato,[10] Sugiyama and Tanaka,[11] Sasaki,[9] and Kuroyanagi.[12] All of these investigators demonstrated the existence of transformation of streptomycin resistance of low degrees by utilizing a strain of *M. avium* highly resistant to streptomycin which was isolated in this laboratory by multiple-step selection of the same strain of *M. avium*, strain Jucho, used in the present study. The strain was distributed to these investigators by the present writers, although they did not point out the correlation between the manner of transformation and the history of the strain.

It has been reported by Hotchkiss[4] that there might be such a phenomenon as linkage between two markers which have been transferred to bacterial cells. The following experiment was made to test the existence of any correlation between isoniazid and streptomycin resistance in the transformation process.

Thirty survival clones on medium containing 0.5 γ of streptomycin were taken at random and subcultured separately. The 30 clones thus obtained were tested for isoniazid resistance by a streak method, inoculating a small size of inoculum onto medium containing no drug and containing 10 γ of isoniazid. The results showed that one of the 30 clones represented as abundant a growth on medium with isoniazid as it did on medium without drug. The remaining 29 clones yielded only a small number of discrete colonies on medium with 10 γ of isoniazid. The results appeared to indicate that most individual cells of one of the 30 clones were a priori resistant to 10 γ of isoniazid. It appears impossible, however, to conclude that such a correlation as linkage may exist between genes determining isoniazid and streptomycin resistance. For, it had been indicated that approximately 4 per cent of the total viable population grown on medium added to the DNA extracted from the resistant culture was resistant to 10 γ of isoniazid. One of the 30 clones, i.e., 3.3 per cent of the clones resistant to 0.5 γ of streptomycin, was also resistant to 10 γ of isoniazid. This does not mean any significant increase of the rate of appearance of isoniazid-resistant mutants in such streptomycin-resistant cells when compared with that in the total viable population of the culture added to the DNA extracted from the resistant culture. Therefore, under the conditions tested, any significant correlation between transformations of isoniazid and of streptomycin resistance could not be found.

In summary: Transformation of isoniazid resistance occurred in a single-step fashion (in all-or-none fashion) in *Mycobacterium avium*, strain Jucho. The results appeared to indicate that isoniazid resistance is produced in the organism

[10] Katsunuma, N., and Nakasato, H.: Kekkaku, 1954, *29*, 19.

[11] Sugiyama, M., and Tanaka, S.: Kekkaku, 1954, *29*, 445.

[12] Kuroyanagi, H.: Personal communication.

by a single gene mutation; this agreed with the results of population analysis studies made previously by the writers.

Transformation of streptomycin resistance is also demonstrated, and the correlation between the history of the strain utilized for the extraction of desoxyribonucleic acid (DNA) and the manner of transformation is discussed.

Under the conditions tested, no significant correlation has been demonstrated between transformations of isoniazid and of streptomycin resistance.

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AN EVALUATION OF CHEMOTHERAPY IN PULMONARY TUBERCULOSIS

High Doses of Isoniazid and PAS versus Daily Streptomycin with High Doses of Isoniazid and PAS

In October, 1956, two pilot studies of two different regimens of antituberculous chemotherapy were begun. Only previously untreated cases of active pulmonary tuberculosis were evaluated. One group of patients received high doses of isoniazid, aminosalicylic acid (PAS), and pyridoxine. A second group received streptomycin daily in addition to high doses of isoniazid, PAS, and pyridoxine.

There have been some recent reports to the effect that drug combinations which incorporate high doses of isoniazid with adequate concentrations of biologically active isoniazid in the blood provide the most effective chemotherapy for pulmonary tuberculosis.[1, 2] Other investigators studying the clinical results which are observed with different combinations of streptomycin, isoniazid, and PAS have reported the following results:

In the cooperative study of the Veterans Administration, Army, and Navy, the results of treatment with isoniazid (300 mg. per day) and PAS (12 gm. per day) were superior to those obtained with streptomycin two or three times weekly with daily PAS or similar streptomycin administration with daily isoniazid (300 mg. per day).[3] British investigators, using isoniazid in doses of 200 mg. daily, reported that a regimen of daily streptomycin and isoniazid was more effective than daily isoniazid–PAS or intermittent streptomycin and isoniazid.[4]

More recently, several investigators have expressed the opinion that the most effective chemotherapy in pulmonary tuberculosis consists of daily streptomycin and high doses of isoniazid with adequate concentrations of biologically active isoniazid in the blood.[1]

The present study was designed primarily to observe the effectiveness of treatment employing high doses of isoniazid along with PAS in comparison with high doses of isoniazid along with both daily streptomycin and PAS.

All patients selected for study had moderately or far advanced pulmonary tuberculosis, and had received either no previous chemotherapy, or chemotherapy for less than two months immediately before admission to the study. Cases of minimal tuberculosis were not studied. Two hundred and sixty patients meeting the above criteria were divided by alternate selection into two treatment groups. One group received isoniazid (10 to 20 mg. per kg. per day), and PAS (12 gm. per day). The other group received streptomycin (1.0 gm. daily for ninety days), isoniazid (10 to 20 mg. per kg. per day), and PAS (12 gm. per day). All patients in both treatment groups also received 100 mg. of pyridoxine daily.

Thirty-three cases were eliminated from the study because of the occurrence of toxic drug reactions, and 22 cases were excluded because of failure to obtain a culture positive for *M. tuberculosis*. This left 113 moderately and far advanced bacteriologically proved cases of pulmonary tuberculosis for evaluation of initial treatment in the high isoniazid and PAS treatment group; and 92 similar cases remained for study in the daily streptomycin, high isoniazid, and PAS treatment group. The dispersion of cases in each treatment group is illustrated in table 1 in terms of extent of disease, presence of cavitation, and type of cavitation.

Sixty-eight of 113 patients in the isoniazid-PAS group had received no previous chemotherapy. The 45 remaining patients had been given drugs immediately before admission for an average of 0.96 months. Forty-two of the 92 patients in the streptomycin–isoniazid–PAS group had not been previously treated, and the remaining 50 patients had received chemotherapy for an average of 0.88 months immediately before admission to the hospital.

The total daily doses of isoniazid which were given in the isoniazid–PAS group were as follows: 21 cases, 10 mg. per kg.; 87 cases, 16 to 18 mg. per kg.; 5 cases, 20 mg. per kg. In the streptomycin–isoniazid–PAS group the total daily doses were: 7 cases, 10 mg. per kg.; 78 cases, 16 to 18 mg. per kg.; 7 cases, 20 mg. per kg. During the first year of study the patients who received 10 mg. of isoniazid per kg. daily were those patients with moderately advanced, noncavitary disease who weighed less than 140 pounds. The remainder were given 16 mg. per kg. on admission. In the second year all patients studied were given 16 mg. per kg. If the value for free isoniazid in the blood was found to be less than 0.3 γ per ml., the daily intake of isoniazid was frequently increased to 18 to 20 mg. per kg. during both years of the study.

The concentrations of biologically active, free isoniazid in the patients' blood were determined in the following manner: One-third of the total daily dose of isoniazid and PAS was administered at 6:00 a.m. (In the triple-drug treatment group streptomycin was discontinued for seventy-two

[1] Kass, I. K., *et al.*: Ann. Int. Med., 1957, *47*, 744.

[2] Berte, S. J., *et al.*: Am. Rev. Tuberc., 1958, *78*, 773.

[3] Livings, D. B.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 20.

[4] Marshall, S. G.: Brit. M. J., 1955, *1*, 435.

TABLE 1

CLASSIFICATION OF CASES

	Isoniazid–PAS Group	Streptomycin–Isoniazid–PAS Group
Total number of cases	113	92
Moderately advanced cases	79.6%	67.5%
Far advanced cases	20.4%	32.5%
Total number of cavitary cases	76.1%	71.7%
Single cavities	50.5%	37%
Multiple cavities	25.6%	34.7%

hours prior to this test.) A light standard breakfast was administered one and one-half to two hours later. Six hours later, at 12:00 noon, venous blood was obtained for analysis. A biologic and chemical method of analysis was utilized in determining the concentrations of free isoniazid in specimens of serum. The reliability of both of these methods has been established in a recent report.[5]

Of the 113 patients who received isoniazid and PAS, the concentrations of free isoniazid in the blood were determined in 100. The values were as follows: less than 0.3 γ per ml. in 7 patients; 0.3 to 0.6 γ per ml. in 15; 0.6 to 9.6 γ per ml. in 78 patients. Serum specimens of 88 of the 92 patients who received streptomycin, isoniazid, and PAS were obtained for determinations of free isoniazid and the values were: less than 0.3 γ per ml. in 6 patients; 0.3 to 0.6 γ per ml. in 11; and 0.6 to 9.6 γ per ml. in 71 patients.

The concentrations of free isoniazid in the blood were considered adequate in nearly all patients in this study. Only about 7 per cent of all patients had values for free isoniazid of less than 0.3 γ per ml., but their disease responded just as well to treatment as did the disease in the 93 per cent with higher concentrations of isoniazid in the blood. Since all patients fared so well, it was not possible to establish any concentration of free isoniazid which was related to the optimal therapeutic results.

Löwenstein-Jensen medium was used as the inoculum for sputum and gastric specimens from all patients throughout the study. After January, 1958, all specimens were inoculated onto an oleic-acid–albumin medium,[6] in addition to the Löwenstein-Jensen medium. Drug-susceptibility studies were not performed on all 205 patients in this report. During the first fourteen months of the study, drug-susceptibility determinations were performed only on cultures of patients who consistently had cultures positive for *M. tuberculosis* after four to five months of treatment. During the last year of the study, the drug susceptibility of tubercle bacilli was determined on all cultures obtained from patients after being admitted to this hospital. The following concentrations of drugs were used in the medium: isoniazid–0.2, 1.0, 5.0, 10.0, and 15.0 γ per ml.; streptomycin–2.5 and 10.0 γ per ml.; PAS–5.0 and 10.0 γ per ml.

Drug toxicity: In 12.7 per cent of 260 patients, it was necessary to discontinue one or more of the drugs used in this study because of drug intolerance (table 2). The types of reactions to each of the drugs were as follows: streptomycin—dizziness, vertigo, rash, fever; isoniazid—rash, fever, hepatitis, psychosis, dizziness, diplopia, headache; PAS—rash, fever, arthralgia, exfoliative dermatitis, and gastrointestinal disturbances which accounted for approximately half of the PAS reactions.

Unfavorable drug reactions were tabulated with relation to the specific drug used and to each of the two multiple-drug treatment groups. Toxicity to streptomycin (10 per cent of 120 patients) was primarily responsible for a 5.6 per cent higher incidence of drug reactions in the triple-drug treatment group. However, the percentage of patients in whom one or more drugs had to be discontinued was only 2.8 per cent higher in the triple-drug group than in the two-drug regimen group. A few patients were successfully desensitized to the offending drug. Undesirable reactions to PAS occurred in 8.5 per cent of those treated, and toxic responses to isoniazid were quite low (3.1 per cent). None of the patients in the study developed peripheral neuropathy due to isoniazid. The administration of 100 mg. of pyridoxine daily

TABLE 2

DRUG-TOXICITY STUDIES

Treatment Group	Number of Patients	Percentage of Drug Reactions	Percentage of Patients with Drug Reactions	Percentage of Patients Excluded for Drug Reactions
Streptomycin–Isoniazid–PAS	120	19.2	17.5	14.2
Isoniazid–PAS	140	13.6	12.9	11.4
All cases	260	16.2	15	12.7

Drug	Number of Patients	Number of Drug Reactions	Percentage of Drug Reactions
Streptomycin	120	12	10.0
Isoniazid	260	8	3.1
PAS	260	22	8.5

[5] BERTE, S. J., *et al.*: Am. Rev. Tuberc., 1959, 79, 344.

[6] Laboratory Methods for Tuberculosis Bacteriology: Report No. 1, 1957, Research and Development Service, Fitzsimons Army Hospital, Denver, Colorado.

appeared to prevent patients from developing peripheral neuropathy.

Reversal of infectiousness: Ninety-nine to one hundred per cent of all 205 patients in both the double- and triple-drug treatment groups had cultures persistently negative for tubercle bacilli after five to six months of observation (table 3). Only one patient (isoniazid–PAS treatment group) did not have persistently negative cultures until seven months of treatment had elapsed. All patients who were lost from the study prior to the five- to six-month observation point had already had cultures negative for *M. tuberculosis* for at least two consecutive months.

The only significant differences in rates of reversal of infectiousness among the various categories of cases listed in table 3 were observed prior to the five- to six-month observation point. After two months of treatment, three-fourths of all patients had produced negative cultures with a difference of only 0.5 per cent between the two treatment groups. Reversal of infectiousness occurred in 21 to 22 per cent more of the moderately advanced than of the far advanced cases at the two months' observation point. A similar situation was observed when a comparison was made between noncavitary and cavitary cases, and between single cavitary and multiple cavitary cases. As would be expected, a higher incidence of negative cultures occurred in the noncavitary and single cavitary cases. The difference in the percentage of patients with cultures negative for tubercle bacilli in each category of each treatment group remained consistently less than 8 per cent and most frequently was less than 5 per cent.

Cavitary closure: Fifty-five to 60 per cent of 152 patients with cavities in both treatment

TABLE 3

BACTERIOLOGIC STUDIES

Types of Cases Studied	Number of Months of Therapy	Isoniazid–PAS		Streptomycin–Isoniazid–PAS	
		Number of Cases	Percentage Negative	Percentage Negative	Number of Cases
All cases	2	113	77.8	78.3	92
	5–6	109	99.0	100.0	88
Moderately advanced	2	90	82.2	85.5	62
	5–6	88	99.0	100.0	59
Far advanced	2	23	60.8	63.3	30
	5–6	21	100.0	100.0	29
Noncavitary	2	27	89.0	96.2	26
	5–6	27	100.0	100.0	23
Cavitary	2	86	72.1	71.2	66
	5–6	82	98.8	100.0	65
Single cavity	2	57	84.2	79.4	34
	5–6	54	98.2	100.0	34
Multiple cavities	2	29	55.1	62.5	32
	5–6	28	100.0	100.0	31

TABLE 4

CAVITY CLOSURE

Types of Cases Studied	Number of Months of Treatment	Isoniazid–PAS Group		Streptomycin–Isoniazid–PAS Group	
		Number of Cases	Percentage with Closed Cavities	Percentage with Closed Cavities	Number of Cases
All cavitary cases	3	86	17.5	24.2	66
	5–6	82	54.8	60.0	65
0.8–2.0 cm. cavities	3	57	26.3	29.0	31
	5–6	55	70.9	71.0	31
2.5–3.5 cm. cavities	3	18	0.0	25.0	24
	5–6	18	50.0	58.3	24
4.0–8.0 cm. cavities	3	11	9.1	18.2	11
	5–6	9	33.3	40.0	10
Single cavities	3	57	26.3	29.4	34
	5–6	54	63.0	70.6	34
Multiple cavities	3	29	3.4	18.8	32
	5–6	28	42.8	48.4	31

TABLE 5

MODERATE TO MARKED ROENTGENOGRAPHIC IMPROVEMENT*

Types of Cases Studied	Number of Months of Treatment	Isoniazid–PAS Group		Streptomycin–Isoniazid–PAS Group	
		Number of Cases	Percentage Improved	Percentage Improved	Number of Cases
All cases	3	113	53.5	59.7	92
	5–6	109	86.2	86.5	88
	8–9	49	96.0	100.0	29
Moderately advanced cases	3	90	61.1	61.3	62
	5–6	88	87.5	83.0	59
	8–9	39	95.0	100.0	18
Far advanced cases	3	23	52.2	56.6	30
	5–6	21	81.0	93.0	29
	8–9	10	100.0	100.0	11

* Moderate to marked improvement represents greater than 50 per cent resolution of abnormal roentgenographic shadows.

groups had closed cavities after five to six months of treatment (table 4). As would be expected, more small cavities and single cavities closed than large cavities and multiple cavities. The highest rate of cavity closure (70.9 to 71.0 per cent) occurred in cavities between 0.8 and 2.0 cm. in size. One of the lowest rates of cavity closure occurred in multiple cavitary cases (42.8 to 48.4 per cent). The rate of closure of cavities of more than 4.0 cm. in size at five to six months was actually the lowest rate (33.3 to 40.0 per cent). However, there were only 11 cases in each treatment group available for study of this point. The rate of closure of cavities after only three months of treatment was extremely variable and never exceeded 30 per cent.

It has been the experience on this hospital service that most cavities that closed as a result of nonsurgical treatment did so within the first five to six months of treatment. In only 4 patients in this study with open cavities after six months of treatment did the cavities close in the seventh and eighth months of treatment. Cavities open after six months of treatment were more commonly found to be still open after nine and twelve months of treatment. The difference in rates of cavity closures in all types of cases tabulated in table 4 suggests that a slightly higher rate of

TABLE 6*A*

DRUG-SUSCEPTIBILITY STUDIES

(Classification of Cases)

Case Number	Extent of Disease	Cavity Present	Catalase Activity	Drug Resistance in Per Cent						
				Streptomycin γ/ml.		Isoniazid γ/ml.				PAS γ/ml.
				2.5	10	0.2	1.0	5.0	10.0	10.0
1	Moderately advanced	Yes, single	Negative	0	0	100	100	100	100	0
2	Far advanced	Yes, multiple	—	0	0	0	0	0	0	100
3	Moderately advanced	Yes, multiple	Positive	20	30	0	0	0	0	0
4	Moderately advanced	Yes, multiple	Negative	10	0	100	100	100	100	0
5	Moderately advanced	Yes, multiple	Negative	100	100	100	100	0	0	0
			Positive	0	0	0	0	0	0	0
6	Far advanced	Yes, multiple	Negative	100	20	100	100	100	100	0
			Positive	0	0	0	0	0	0	0
7	Far advanced	Yes, multiple	Negative	0	0	100	100	100	100	0

TABLE 6*B*

DRUG-SUSCEPTIBILITY STUDIES

(Response to Treatment)

Case Number	Number of Months to Culture Negativity	Number of Months to Cavity Closure	Roentgenographic Improvement			Comments
			3 Months	6 Months	9 Months	
1	1	3	Moderate	Moderate	Moderate	Isoniazid concentration, 0.3–0.6 γ/ml.
2	1	6	Slight	Moderate	Moderate	Isoniazid concentration, 0.6–1.2 γ/ml.
3	1	4	Slight	Moderate		Streptomycin stopped after 90 days
4	2	5	Moderate	Moderate		Streptomycin for 2 weeks; dihydrostreptomycin thereafter
5	2	Open	Moderate	Moderate		Streptomycin stopped after 90 days
6	4	Open	Worse	Moderate		Streptomycin continued after 90 days
7	2	5	Moderate	Moderate		Streptomycin continued after 90 days

closure occurred in the triple-drug treatment group. However, a closer analysis reveals the differences to be no greater than 8.3 per cent in any category at the five- to six-month observation point. With the number of cases reported herein and the differences between the treatment groups, one cannot definitely justify an impression that daily streptomycin along with high doses of isoniazid and PAS provides a superior form of treatment to that provided by high doses of isoniazid and PAS alone.

The criteria for determining cavity closure included planigraphic study. In 5 cases the cavities appeared to have been closed on conventional roentgenograms, but were observed to be open cavities on planigrams. These cases were recorded as open cavities in this study.

Roentgenographic improvement: Approximately 85 per cent of all patients in both groups demonstrated moderate to marked roentgenographic improvement after five to six months of treatment (table 5). Ninety-five to 100 per cent of all patients showed significant roentgenographic improvement after eight to nine months of treatment. The roentgenographic findings in 2 patients became worse in three months, but revealed moderate improvement after six months. In a comparison of all cases, moderately advanced cases and far advanced cases in both double- and triple-drug treatment groups, there appears to be no significant difference in the degree of roentgenographic improvement.

Drug susceptibility: In *Cases 1*, *2*, and *3* (tables 6*A* and 6*B*), the only culture of tubercle bacilli obtained occurred immediately after admission to the hospital. In *Case 7*, the admission culture contained organisms resistant to isoniazid. The last culture occurred in the second month of treatment, but no susceptibility tests were performed on this latter culture. Admission cultures were found to be drug resistant in *Cases 5* and *6*. However, cultures after one and two months of drug therapy, respectively, revealed the presence of organisms which were fully susceptible to all drugs. In *Case 4*, the only culture obtained occurred after one month of therapy. In this case, no previous streptomycin therapy had been administered.

Seven patients were found to have drug-resistant tubercle bacilli during the last half of the study when drug-susceptibility tests were performed routinely. The data on these 7 cases are summarized in tables 6*A* and 6*B*. In *Cases 5* and *6*, the patient excreted a drug-susceptible, catalase-positive organism in addition to a drug-resistant mutant. In *Case 1*, it is suspected that an isoniazid-susceptible strain of tubercle bacilli was also present within the patient but was not isolated on culture. In *Case 4*, the patient developed labyrinthitis after two weeks of streptomycin therapy, and dihydrostreptomycin was substituted. Streptomycin was continued beyond a ninety-day period in *Cases 6* and *7*. It is of interest to note that all 7 of these patients had never received any previous chemotherapy for tuberculosis except the patient of *Case 5*, who had received isoniazid and PAS for ten days before admission to the hospital.[7]

In summary: One hundred and thirteen patients with moderately and far advanced bacteriologically proved active pulmonary tuberculosis were given initial treatment with high doses of isoniazid, PAS, and pyridoxine. Ninety-two patients with similar disease were treated with daily streptomycin, in addition to high doses of isoniazid, PAS, and pyridoxine. The concentrations of biologically active, free isoniazid found in the blood were considered adequate in the majority of all 205 cases. An analysis of treatment in all cases revealed the following: When high doses of isoniazid along with PAS are used in the treatment of pulmonary tuberculosis, and when adequate concentrations of free isoniazid are obtained, the use of daily streptomycin for ninety days, in addition to the other drugs, does not provide any significantly superior therapeutic results.

Seven patients excreted tubercle bacilli which were noted to be resistant to one or more drugs. Despite these findings, all 7 patients demonstrated very satisfactory responses to treatment and early reversal of infectiousness.

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[7] There is one additional case of drug resistance that has recently been found. The patient had moderately advanced cavitary tuberculosis and was in the triple-drug treatment group. The concentration of free isoniazid in the blood six hours after drug ingestion was 0.6 to 1.2 γ per ml. Admission cultures were fully susceptible to all three drugs. After one month of treatment, all gastric and sputum cultures continued to be negative for tubercle bacilli. The patient was subjected to surgical resection for persistent cavitation after six months of chemotherapy. Drug-susceptibility studies on the tubercle bacilli isolated from the resected lung specimen showed the following: At concentrations of isoniazid of 0.2, 1.0, 5.0, and 10.0 γ per ml., the percentage of resistant organisms was, respectively, 100, 40, 60, and 70. Resistance to 2.5 and 5.0 γ per ml. of streptomycin was, respectively, 30 and 0 per cent. Resistance to 10γ per ml. of PAS was 100 per cent. This organism was catalase negative.

FURTHER STUDIES ON THE SPOT TEST FOR THE DIFFERENTIATION OF TUBERCLE BACILLI OF HUMAN ORIGIN FROM OTHER MYCOBACTERIA

Distinguishing tubercle bacilli of human origin from other strains of mycobacteria isolated from pathologic material was based until recently only on cultural and pathogenic characteristics. Such methods are laborious, time-consuming, and expensive; moreover, inconsistent results leading to confusion are frequently obtained. The chemical procedures recently proposed by Konno,[1, 2] based on the abundant production of niacin by *Mycobacterium tuberculosis* as compared with all other mycobacteria, constitute a significant contribution leading to the simplification and accuracy of this differentiation. Unfortunately, Konno's tests have several limitations: the direct test (performed with colonies of bacilli) often provides inconsistent or obscure results, and the transfer of several loopfuls of mycobacterial growth to a tube for each test is a bothersome procedure when the number of strains to be tested is large; the reliable indirect test (performed in a synthetic liquid culture) is a more elaborate and time-consuming procedure.

Trying to obviate these limitations, the spot test for the differentiation of human tubercle bacilli from other mycobacteria was devised in this laboratory[3]; this test gives reproducible and consistent results, eliminates the limitations of Konno's procedures, and implies a 95 per cent saving for each reagent. Independently, Runyon and associates[4] have proposed more recently a modified procedure based on a similar principle, i.e., the extraction of niacin from cultures on solid medium.

The purpose of the present paper is to provide additional evidence on the accuracy and reliability of the spot-test method as a differential procedure, and to present results on the effects of pH on the extraction of niacin from cultures, on the reaction itself, as well as the effects of storage of the cultures to be tested.

Spot-test method: The technique is essentially the same as previously described[3]: To a tube of a well-grown culture on Löwenstein-Jensen medium of the strain to be tested, 0.5 to 0.8 ml. of distilled water was added, and the tube was sterilized in the autoclave at 15 pounds for fifteen minutes; the tube was then cooled in a water bath; 2 to 4 drops of the extract thus obtained[5] were permitted to fall directly from the tube to a depression of a porcelain spot-test plate, and then one 0.05-ml. drop of a 1.5 per cent weight/volume alcoholic solution of *o*-tolidine and one drop of a 10.0 per cent weight/volume aqueous solution of cyanogen bromide were added; the plate was gently rotated several times, and readings made four or more minutes later. Human strains of tubercle bacilli developed a red coral color (3-plus to 4-plus reaction), whereas all other mycobacteria tested had either no color or were a light blue (negative reaction) or a light dirty pink (doubtful reaction).

Spot test with recently isolated and standard laboratory strains: The test was carried out with duplicate cultures of the following organisms: 52 strains of *M. tuberculosis* isolated from sputum, gastric contents, urine, cerebrospinal fluid, and surgical specimens, all from different human pa-

TABLE 1

SPOT TEST FOR NIACIN WITH RECENTLY ISOLATED AND STANDARD LABORATORY STRAINS OF MYCOBACTERIA

Strains Tested	Results of the Test
M. tuberculosis:	
52 strains from patients	+++ to ++++
H37Rv	++++
R1Rv	+++
M. bovis:	
Ravenel	±
Sáenz	−
4228-2	±
4228-3	±
4228-4	±
D-4	±
Cornell 47642	±
BCG Phipps	−
BCG Moreau	−
BCG London	−
M. avium:	
Kerchberg	−
4109-2	−
Robert Koch Institute	−
Unclassified mycobacteria:	
1 photochromogen	−
5 scotochromogens	−
4 nonpigmented smooth slow-growers	−
Saprophytic mycobacteria:	
M. phlei w	±
Mycobacterium sp. 607	±
Mycobacterium sp. S-701	±

[1] KONNO, K.: Science, 1956, *124*, 985.

[2] KONNO, K., *et al.*: Am. Rev. Tuberc., 1957, *75*, 529.

[3] GUTIERREZ-VAZQUEZ, J. M.: Rev. lat.-amer. Microbiol., 1958, *1*, 287.

[4] RUNYON, E. H., *et al.*: Am. Rev. Tuberc., 1959, 79, 663.

[5] It seems clear now that it is unnecessary to measure accurately the volume of extract to be employed, providing it is large enough.

TABLE 2

EFFECTS OF DIFFERENT VALUES OF pH ON THE SPOT-TEST REACTION CARRIED OUT WITH FILTRATES OF H37Rv STRAIN OF MYCOBACTERIUM TUBERCULOSIS

Values of pH	Results of Spot Test
2.60	±
2.80	±
3.00	+
3.30	+
3.90	++
4.35	+++
4.65	+++
5.00	+++
5.20	+++
5.60	+++
5.80	+++
6.15	+++
6.50	+++
7.10	++++
7.35	++++
7.95	++++
8.40	++++
9.00	++++
10.15	+++

TABLE 3

EFFECTS OF DIFFERENT VALUES OF pH ON THE EXTRACTION OF NIACIN FROM CULTURES OF THE H37Rv STRAIN OF M. TUBERCULOSIS ON LÖWENSTEIN-JENSEN MEDIUM AS DETERMINED BY THE SPOT-TEST METHOD

Values of pH at Which Extraction Was Carried Out	Results of Spot Test
3.0	++++
4.0	++++
5.0	++++
6.0	++++
7.0	++++
8.0	++++
9.0	++++

tients; 10 strains of "atypical" mycobacteria also isolated from patients (one photochromogen, 5 scotochromogens, and 4 nonpigmented smooth slow-growers); 2 standard strains of *M. tuberculosis* (H37Rv and R1Rv); 10 standard strains of *M. bovis* (Ravenel, Sáenz, 4228-2, 4228-3, 4228-4, D-4, Cornell 47642, BCG Phipps, BCG Moreau, and BCG London); 3 standard strains of *M. avium* (Kerchberg, 4109-2 and Robert Koch Institute); and 3 strains of saprophytes (ATCC 607, *M. phlei* w,[6] and S-701, a chromogen from soil).

[6] This strain was labeled as such when received from the collection of our institution. It was originally sent by Dr. S. A. Waksman of Rutgers University.

The results of these tests appear in table 1. As may be seen, only human tubercle bacilli gave an intense (3-plus to 4-plus) reaction, the results for all other strains being doubtful or completely negative.

The effects of different values of pH on the reaction: A sterile culture filtrate giving a strongly positive reaction for niacin was obtained from a well-grown culture of the H37Rv strain of *M. tuberculosis* on Proskauer and Beck medium; the pH of several aliquots from this filtrate was adjusted to values ranging from 2.6 to 10.15, as determined in a Model G Beckman potentiometer, by adding very small amounts of 4N solutions of hydrochloric acid or sodium hydroxide. The spot test was repeatedly carried out with these aliquots in order to establish the influence of pH on the reaction.

As may be seen in table 2, the results are affected only by values of pH too low to be encountered in routine cultures (2.6 to 3.0). The colors obtained were deep when the reaction was carried out from pH 4.35 to pH 10.15.

Effects of different values of pH on the extraction of niacin from cultures on Löwenstein-Jensen medium: Once it was determined that a wide range of pH values did not interfere with the reaction itself, it was ascertained whether the extraction of niacin from the cultures was not impaired by extreme values of pH. To accomplish this, buffer solutions according to Clark and Lubs were used instead of distilled water for extraction in the autoclave. Tubes of well-grown cultures of the H37Rv strain of *M. tuberculosis* on Löwenstein-Jensen medium were employed. The spot test was carried out with extracts thus obtained.

Results in table 3 show that enough niacin from the growth and the medium could be extracted at values of pH ranging from 3.0 to 9.0 to provide a 4-plus reaction.

Spot test in stored cultures: In order to establish whether the method could be performed with old stored cultures, the spot test was carried out with tubes of several of the above mentioned strains, grown on Löwenstein-Jensen medium and having variable periods of storage at 4°C.; the periods of storage ranged from none to more than 250–300 days (some of the cultures were completely dry).

As may be seen in table 4, human strains of tubercle bacilli give 3-plus to 4-plus reactions irrespective of the period of storage or the dryness of the cultures; other mycobacteria give consistent negative to doubtful reactions in the same conditions, while a few others (Sáenz strain of *M. bovis*, and S-701 and PT-SIM-903 strains of *Mycobacterium* sp.) change from negative to doubtful or to one-plus reaction as the time of storage increases; nevertheless, there still exists a clear-cut difference between *M. tuberculosis* of human origin and all other mycobacteria under these conditions.

TABLE 4

SPOT TEST IN STORED CULTURES

Strains Tested	Time of Storage	Results of Spot Test
	days	
M. tuberculosis H37Rv	None	++++
	63	+++
	130	+++
	246	+++
	324	++++
M. tuberculosis R1Rv	None	+++
	62	+++
	100	+++
	200	+++
M. tuberculosis 58182 (isolated from sputum)	None	+++
	43	+++
	85	++++
	200	++++
	237	++++
M. tuberculosis 58184 (isolated from sputum)	None	+++
	30	+++
	106	+++
	214	++++
	239	+++
M. tuberculosis 58186 (isolated from urine)	None	++++
	44	++++
	97	++++
	228	+++
	271	++++
M. tuberculosis 58217 (isolated from gastric contents)	None	+++
	43	+++
	114	++++
	216	++++
	245	++++
M. tuberculosis 58353 (isolated from sputum)	None	+++
	24	+++
	39	+++
	64	++++
	123	++++
M. bovis BCG Phipps	None	−
	14	−
	30	−
	61	−
	87	−
M. bovis Sáenz	None	−
	32	−
	245	+
M. avium 4109-2	None	−
	23	−
	42	−
	98	−
Mycobacterium sp. 607	None	±
	18	±
	95	±
Mycobacterium sp. S-701 (a chromogen from soil)	None	−
	83	−
	167	−
	241	±
	258	+

TABLE 4—*Continued*

Strains Tested	Time of Storage	Results of Spot Test
	days	
M. phlei w	None	±
	84	±
	137	±
	233	±
Mycobacterium sp. PT-SIM-903 (a scotochromogen from sputum)	None	−
	7	−
	89	−
	167	−
	233	±
	261	±
Mycobacterium sp. PH-562 (a nonpigmented smooth slow-grower from sputum)	None	−
	25	−
	43	−
	98	−
	130	−

The spot test as proposed is a simple, rapid, and easily reproducible method; it is completely safe, as the cultures are sterilized during the first step (extraction); false negative, doubtful, or weak reactions are eliminated for human tubercle bacilli perhaps because a better extraction is accomplished at high temperatures and/or because under these conditions more niacin is released from precursors. The reaction seems free from the influence of the age of the culture or its pH and could be performed in completely dried-up cultures. By extracting the cultures as described, little if any growth goes with the extract, even in the case of smooth chromogenic strains. Furthermore, the pigment of these strains or the dye from the medium does not interfere with the observation of the presence or absence of the coral red color developed in the reaction.

In summary: The spot test for distinguishing *M. tuberculosis* of human origin from other mycobacteria has continued to give consistent and reproducible results. The results are clear-cut even with old stored and with completely dried-up cultures. Variation in pH values from 3.0 to 9.0 apparently does not affect final results.

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[7] The writer wishes to acknowledge the kindness of Drs. William Steenken, Jr., José López Vallejo, and Luis F. Bojalil, for sending to this laboratory some of the standard strains tested.

THE TREATMENT OF TUBERCULOSIS WITH ALTERNATING DRUGS

Five years ago, in the spring of 1954, the writer was unhappy about the treatment failures which continued to occur—and by all reports are still a common experience among those who work with tuberculosis. It was recalled that no one had suggested that resistance to any of the drugs developed under approximately three weeks, even when one of the drugs was used alone. At that time there were four antituberculous drugs available. The question arose as to what would happen if two drugs were used together for not longer than one month and were then followed by the use of two different drugs the following month. Might not the tubercle bacilli be so affected that resistant cells would not emerge, the effects of the drug might be prolonged indefinitely, and no treatment failures would occur? As we have come to recognize the different modes of action of the various drugs on tubercle bacilli, this assumption has appeared less unreasonable. The remainder of this paper represents an attempt to provide affirmation of this concept.

An important influence on the writer's thinking on the subject was provided by the previous experience with syphilis. When Moore and Keidel[1] of Johns Hopkins published their material on alternating drugs in the treatment of that disease, they pointed out that continuous treatment with the same drugs was impossible because of two things: first, the development of patient intolerance; and, second, the development of resistance by the treponemes. These are also the problems in treating tuberculosis. Moore and Keidel also pointed out the dangers of intermittent therapy. Their solution was alternating drugs, and they stated: "This type of continuous treatment with alternating courses . . . may be continued indefinitely without detriment to the patient." They added: "Inasmuch as there is no way of differentiating the potential cure from the potential failure before treatment is begun, it seemed certain that the only safe procedure was to treat every patient up to the point necessary to cure the most resistant member of the group."

In the last few years, reports of "rotating" drug regimens have been published by Tanner in 1953,[2] by Joiner and associates in 1956,[3] and by Calix and White in 1956.[4] All have pointed out the superior results of this form of treatment.

The regimen used in the present observations has been somewhat different. In 1954, pyrazinamide was available on an experimental basis. The writer had used some of this and, with many others, had been troubled by its toxicity.[5-11] The possibility was considered, however, that pyrazinamide might show less toxicity when used for shorter periods. Because of this and because of the situation with respect to drug resistance as outlined above, a schedule of therapy was set up as follows:

Streptomycin, 1 gm., was given three times a week with 3.0 gm. of PAS four times daily for the first month, and was alternated on a monthly basis with 100 mg. of isoniazid four times daily and 1.0 gm. of pyrazinamide three times daily. This alternating regimen has been continued with certain modifications ever since in all cases under discussion.

To ease the administrative problems for the nurses, the drugs were alternated on a calendar month basis, so that patients were begun on either streptomycin–PAS or pyrazinamide–isoniazid, depending on when they were admitted to the hospital.

The dose of streptomycin for the first month (or, if streptomycin for less than two weeks was given on the first course, for the second course as well) was increased to 1.0 gm. daily.

If any intolerance developed, cycloserine was substituted for the offending drug and the alternating schedule thus modified was continued. Very occasionally, oxytetracycline or viomycin was used in this way when there was intolerance (or, in the case of re-treatment, drug-resistant tubercle bacilli to more than one drug).

In a very few of the most severe cases recently, cycloserine has been added as a third drug during the first month or two of therapy.

[1] Moore, J. E., and Keidel, A.: Bull. Johns Hopkins Hosp., 1926, *39*, 1.

[2] Tanner, E., *et al.*: Schweiz. med. Wchnschr., 1953, *83*, 751.

[3] Joiner, C. L., *et al.*: Lancet, 1956, *2*, 165.

[4] Calix, A. A., and White, K.: J. M. A. Alabama, 1956, p. 3.

[5] McDermott, W., *et al.*: Am. Rev. Tubere., 1954, *69*, 319.

[6] Muschenheim, C., *et al.*: Am. Rev. Tubere., 1954, *70*, 743.

[7] Potter, B. P., and Chang, S. F.: Dis. Chest, 1955, *27*, 44.

[8] Phillips, S., and Horton, G. E.: Am. Rev. Tubere., 1956, *73*, 704.

[9] Quarterly Progress Report of the Veterans Administration, Army, and Navy Study on the Chemotherapy of Tuberculosis, 1955, *10*, 81.

[10] American Trudeau Society: Am. Rev. Tubere., 1957, *75*, 1012.

[11] Schwartz, W. S., and Moyer, R. E.: Am. Rev. Tubere., 1957, *76*, 1097.

The first patient was begun on this treatment on April 24, 1954, and 3 others were added shortly after. At the end of August, 1954, pyrazinamide suddenly became unobtainable and at that time there were no satisfactory substitutes available, so the alternating regimens had to cease. Although these 4 patients, treated in this way for so short a time, proved nothing, sputum "conversion," in spite of the fact that patients with severe disease had been deliberately chosen, was considered remarkable. The failure of the tubercle bacilli to reappear in the sputum in the 3 most severe cases after the first four months was likewise remarkable. It appeared that the regimen had promise. The writer began to wonder whether there was really as much time available for the proper treatment of a tuberculous infection as was generally thought, and whether the usual leisurely pace of the first few months of therapy might not be quite wrong. One could not help suspect that these unexpected successes were due to unusually energetic treatment in the first two months of therapy.

During the summer of 1955, 4 more cases were added, all seriously ill patients. Pyrazinamide was still not available, but oxytetracycline was used in its place. These patients did well, too, so, when cycloserine became available in May, 1956,[12] and pyrazinamide again in August, 1956,[13] the monthly alternating regimen was restarted. Pyrazinamide was continued in the regimen routinely to find out something of the toxicity of pyrazinamide used in this way; also because it had been suggested that isoniazid–pyrazinamide were particularly effective when used together.[4]

The results presented herein were obtained in 83 previously untreated patients with pulmonary tuberculosis, all of whom had been treated for at least six months on an alternative regimen, except the first 4 original patients who had received somewhat shorter chemotherapy. The latter are included for no better reason than historic interest. No more patients were admitted to the study after July 15, 1958, so that there has been a maximal period of observation of five years and a minimal period of ten months. At first, the more severe cases were treated on this regimen, then gradually all of the patients admitted to the male ward, and (since about the beginning of 1958) most of the admissions to both male and female wards. There is, therefore, a preponderance of males in the series (64 males and 19 females). The average age was forty-one years; the oldest was eighty-three, the youngest sixteen. They appeared to be a reasonable cross section of the community. At least one had a frank psychiatric problem. All were treated in the hospital while on the alternating regimen. Usually isoniazid and PAS were continued at home after discharge for a varying period up to a total of two years on drugs (including the period of treatment in the hospital).

Examinations after discharge from the hospital have been made in the outpatient department. While in the hospital, sputum (or gastric contents) has been examined by microscopy and culture three or four times on admission and once a month thereafter in the hospital. After discharge, sputum or gastric smears and cultures have been done once a month in the Provincial Laboratory in Toronto.

Of these 83 patients, 6 had minimal disease, 51 moderately advanced, 19 far advanced, and 7 very far advanced disease. These adhere to the National Tuberculosis Association classification except for the very far advanced group, a group which may be unrecognized by the National Tuberculosis Association officially but which is easily recognizable by every phthisiologist.

The complications were familiar: 9 had diabetes (4 of these with far advanced disease); 5 had bronchiectasis, chronic bronchitis, or emphysema along with their tuberculosis; 3 had significant arteriosclerosis or heart disease; one had silicosis; and one had hydatid disease.

Of the total, 65 had tubercle bacilli in sputum or gastric washings at some time during observation. In 41 patients, the sputum contained tubercle bacilli only before treatment or during the first month of treatment, but not thereafter. In 9 patients the sputum was infectious after one month of treatment but not thereafter; in 5 it remained infectious after two months of treatment; in 4, after three months of treatment; and in 5, tubercle bacilli were still present after four months of treatment. The sputum remained noninfectious thereafter in all but one patient in whom reversal of infectiousness required seven months. The last-named patient was one of the 4 in the original series begun in 1954. The alternating regimen had to be discontinued after only three months of this form of treatment because of the lack of pyrazinamide. The patient's sputum became negative for tubercle bacilli in July, 1954, but became positive again *once only* in January, 1955.

Seventeen nonpulmonary primary-treatment patients were also treated in this way. The number is too small to be significant. It may be stated, however, that the results were quite satisfactory and in substantial agreement with the findings in the pulmonary cases.

The writer now believes that no patient with tuberculosis, no matter how much disease is present, need be discharging tubercle bacilli in

[12] Received through the courtesy of Merck Sharp & Dohme.

[13] Received through the courtesy of Frank Horner Co. of Canada.

the sputum after four months of treatment, and no relapse should occur. Naturally, this conclusion will have to be re-examined as more cases are studied.

It is not possible to give any meaningful report on the resected specimens, and this is most unfortunate. The specimens were subjected to the vagaries of student pathologists who rotated frequently. The reports, therefore, are not consistent. Thirty-two resections were done. All of the patients were noninfectious for several months before surgery—in most cases for periods of six to eight months.

Fifty more patients were treated on an alternating regimen, but they are excluded from the hundred already reported because: other antituberculous drugs had been used before the alternating regimen was begun (either on a previous hospital admission or during the current admission); or the diagnosis of tuberculosis was proved to have been wrong. This occurred in 4 cases (one bronchiectasis, one carcinoma, 2 fungal disease). The 50 cases are added to the group already presented in order to discuss drug toxicity.

With respect to drug toxicity in this group of 150 cases, it might be helpful to note first the length of time the patients were on this course of treatment. One hundred and three patients were treated for six months to one year (including the 4 original patients treated for slightly less than six months), 45 patients were treated for one to two years, and 2 patients have been treated for slightly more than two years continuously. It might be noted that these last 2 have required no revision whatever of their original alternating regimen, suggesting that drug intolerance is related more to the individual patient and his idiosyncrasies than to the length of the chemotherapy.

Streptomycin produced the most difficulty, requiring substitution in 22 cases. The PAS required substitution in 15 cases, cycloserine in 9, pyrazinamide in 7, isoniazid in 3, and oxytetracycline in one case.

It should be noted that pyrazinamide was not stopped in a single case because of liver damage. Cycloserine, which was used very much less frequently, offended more often.

It must be confessed that bromsulphalein tests of liver function were not made routinely because of a lack of sufficient personnel in the laboratory to follow this many cases with this procedure. A cephalin cholesterol flocculation was performed on each patient at the end of each month of pyrazinamide treatment. It is recognized that this is not a satisfactory gauge of liver toxicity when dealing with this drug. In a very few cases the cephalin cholesterol flocculation did show increased reaction but, as there was no clinical evidence of toxicity, the regimen was continued. Through subsequent months of pyrazinamide therapy, the values for the cephalin cholesterol flocculation returned to normal.

Recently, bromsulphalein tests were performed on 38 patients who had been on the alternating regimen for at least six months. In only 4 of these patients was more than 5 per cent dye retained after forty-five minutes (the most was 9 per cent), and 2 of these patients were more than sixty-five years of age. All of the values were well below the 25 per cent dye retention considered by Phillips and Horton to be premonitory of liver disease.[5] Certainly more work is required on this matter, but it should be emphasized that not a single case of jaundice has occurred so far, and no other clinical evidence of liver damage has appeared.

Some writers have suggested that there is a special danger in using pyrazinamide in alcoholics. Fourteen of the 150 patients were alcoholics (8 of the 14 were re-treatment cases), yet they encountered no difficulty. If one were a little less charitable in the use of the term "alcoholic," it is possible that the group might be considerably larger.

Roentgenographic clearing has been good—in some cases remarkable—but it is believed that attention should be primarily directed to the sputum "conversion" and absence of relapse rather than roentgenographic clearing. The last-named is difficult to assess and has been produced satisfactorily on other regimens.

Of the 100 previously untreated patients, 68 are back at work, 14 others have left the hospital, and 2 are dead (both deaths were unrelated to spread of tuberculosis or its medical management).

It should be stressed that the writer is not suggesting the use of any particular drug, but he *is* suggesting that (*1*): Effective drugs be used in full therapeutic doses; the regimen should not be commenced in re-treatment cases until drug-susceptibility studies have been reported. (*2*) These effective drugs should be used in such a way that two drugs are used by monthly alternation with two different drugs (A and B, then C and D; then A and B, et cetera). (*3*) Finally, in this connection, it is believed that this regimen should be continued until the maximal clearing has taken place, as shown by stable roentgenographic findings.

In summary: The results have been presented of the treatment of 100 tuberculous patients on a regimen of two-drug pairings alternated by months. It is believed that on the alternate-month regimens drug toxicity can be kept to a minimum, the emergence of drug-resistant tubercle bacilli can be suppressed, and it is easier to keep patients in the hospital because of the psychologic effects

of such varied therapy. It should be stressed that the first few months of treatment are of the greatest importance. There is not endless time to spar and feint with tuberculosis. What is needed is not new drugs, but the wise use of what we have and a refusal to shrug off failures as things which must be.

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ISOLATION OF LYSOGENIC ACID-FAST BACILLI FROM THE SOIL

Mycobacteriophages have been isolated from soil samples by several investigators. The existence of lysogenic mycobacteria in the soil may be responsible for the isolation of these mycobacteriophages, because examples of lysogeny among Streptomyces isolated from soil samples have been reported by Bradley.[1] However, no example of naturally lysogenic mycobacteria has been obtained, although efforts were made by the present writers to detect lysogenic strains among many laboratory strains of mycobacteria[2] and among more than one hundred human strains newly isolated from tuberculous patients.[3]

Acid-fast bacilli were isolated from soil samples by the use of the Söhngen medium.[4] These bacilli were differentiated from acid-fast Nocardia and were identified as Mycobacterium through their growth characteristics. The bacterial culture was inoculated on a thin layer of 4 per cent glycerol agar deposited on a slide glass, covered with a cover glass, and incubated at 37°C. Observations with the phase contrast microscope revealed that the bacilli did not branch but grew in the characteristic way of mycobacteria. Several strains of 22 isolated mycobacteria were found to release free phage particles in their culture filtrates. One of the strains was purified by fifteen serial replatings from well-isolated colonies on a 4 per cent glycerol agar plate. A single colony of this strain was further inoculated in Dubos broth base medium for twelve generations, and a number of free phage particles in the supernatant were counted by the agar layer method for each generation. This strain was designated as 3-A. The 3-B strain, which was also isolated from the same soil sample, was used as the indicator strain.

The result is shown in table 1. The number of free phage particles per milliliter of the supernatant for each generation was approximately constant, being 10^9 in order. Since Tween® 80 contained in Dubos broth base medium is known to prevent the adsorption of mycobacteriophage to mycobacteria, virulent phages, if present, would be unable to multiply and would rather decrease in number during successive cultivation in this medium. Therefore, the result obtained above will indicate that this strain is not a "carrier-strain" but is lysogenic. The plaque type of the released phage on the indicator strain 3-B is shown in figure 1. This phage, designated as R3A, was active against 8 of 22 strains of mycobacteria isolated from different soil samples but not lytic against several laboratory mycobacterial strains so far tested. An electron micrograph of an air-dried specimen of this phage is presented in

TABLE 1

NUMBER OF PLAQUES PER MILLILITER OF CULTURE FILTRATE OF 3-A STRAIN IN DUBOS BROTH BASE MEDIUM

	Generations in Dubos Broth Base Medium								
	3	4	5	7	8	9	10	11	12
Number of free phage particles	2×10^9	9×10^9	4×10^9	6×10^9	8×10^9	6×10^9	5×10^9	1×10^9	3×10^9

FIG. 1. Plaque type of mycobacteriophage R3A on indicator strain 3-B.

[1] BRADLEY, S. G.: Science, 1957, *126*, 558.

[2] TAKEYA, K., *et al.*: Am. Rev. Resp. Dis., 1959, *80*, 543.

[3] TAKEYA, K., *et al.*: Unpublished data.

[4] SÖHNGEN, N. L.: Zentralbl. Bakt., 1913, *37*, 595.

FIG. 2. Electron micrograph of the temperate phages R3A released from lysogenic strain 3-A.

figure 2. The phage was revealed to be tadpole-shaped with an extremely long tail.

Although artificial lysogenization of mycobacteria has recently been reported,[2] a naturally lysogenic strain has not yet been obtained. The detection of the naturally lysogenic strain of mycobacteria in this experiment suggests a probable origin of mycobacteriophages isolated by a soil enrichment technique and, on the other hand, indicates that the well-known relationship between bacteriophage and genetics can also be applied to mycobacteria. On further investigation, many strains not now considered lysogenic will probably be found to contain temperate phages.

In summary: Free phage particles were found in the culture filtrates of several strains of mycobacteria isolated from soil samples. One of these strains was further examined and confirmed as a naturally lysogenic strain. The temperate phage released from this strain was lytic against 8 of 22 strains of mycobacteria isolated from soil samples, but not lytic against several laboratory strains so far examined.

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REDUCTION OF CHRONICITY IN PULMONARY TUBERCULOSIS BY PREDNISONE

Despite modern effective chemotherapeutic combinations that are employed in active tuberculosis, about 20 per cent of the patients fail to respond favorably.[1] This failure is usually due to factors peculiar to the patient himself, such as low resistance, late detection, personality makeup, et cetera. The disease of these patients eventually becomes chronic and they manage to survive, becoming health hazards when they live outside institutions, and economic liabilities when living within. The present study was initiated to determine the effectiveness of small doses of prednisone in a group of potentially chronic patients.

Fifty-two such patients were chosen and divided into two equal groups. The first group of 26 patients served as controls and the second group received prednisone[2] (table 1). All patients selected had had a minimum of four months of antimicrobial therapy with no significant improvement. The criteria for this selection were: no perceptible roentgenographic improvement, persistence of tubercle bacilli in the sputum, and either clinical deterioration or no change. It is conceivable that some of these patients at the four-month period were about to show some favorable response.

The age of the patients varied from sixteen to sixty-two years with an average age of thirty-nine years. Sixteen patients were men and the remaining 10 patients were women. There were 16 nonwhites, 10 whites. Roentgenograms taken before the treatment showed that 16 patients had far advanced disease and 10, moderately advanced. The sputum of all of the 26 patients contained tubercle bacilli. Five patients suffered from severe toxemia and were bedridden.

All patients were given 10 mg. of prednisone daily in two equally divided doses. Their antimicrobial drugs were unchanged so that the only additional factor that was introduced into the regimen was prednisone. The antituberculous drugs used were isoniazid-PAS and isoniazid-cycloserine.

Twenty-six patients have received the drug to date from four to nine months continuously with no evidence of toxicity.[1] This is in contrast to the previous report from the present writers, in which it was noted that larger doses of prednisone produced some undesirable side effects.[3] Clinical improvement was marked soon after therapy in all patients. All of the 5 patients who had had toxemia and were previously bedridden improved and became ambulatory. The bacteriologic data on these 26 patients as compared with the control group are given in table 2. It is evident that the first group has shown decided progress toward sterilization of the sputum. The data from serial roentgenograms of the chest from both groups are given in table 3. These are also indicative of the distinct advantage of the therapy received by the first group. Two patients in the prednisone-treated group have become worse roentgenographically, whereas there was only one worsening

TABLE 1

BACKGROUND DATA ON PREDNISONE-TREATED AND UNTREATED PATIENTS

	Prednisone-treated Patients	Control Patients
Sex	16 male—10 female	18 male—8 female
Race	16 nonwhite—10 white	11 nonwhite—15 white
Roentgenographic findings	16 far advanced disease; 10 moderately advanced disease	15 far advanced disease; 11 moderately advanced disease

TABLE 2

BACTERIOLOGIC DATA ON PREDNISONE-TREATED AND UNTREATED PATIENTS

Weeks	Number Observed		Positive for Tubercle Bacilli		Negative for Tubercle Bacilli	
	Pr	Con	Pr	Con	Pr	Con
4–6	26	26	6	26	20	0
6–12	26	25	2	24	24	1
12–24	17	18	4	16	13	2
24–36	11	13	2	11	9	2

Pr: Prednisone group.
Con: Control group.

[1] LIVINGS, D. G.: Tr. Eighteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1959, p. 18.

[2] Prednisone used for this study was kindly supplied by Dr. E. Henderson of the Schering Corporation, Bloomfield, New Jersey.

[3] EPSTEIN, I. G., *et al.*: Dis. Chest, 1959, *35*, 509.

TABLE 3

ROENTGENOGRAPHIC DATA ON PREDNISONE-TREATED AND UNTREATED PATIENTS

Weeks	Number Observed		No Change		Moderate		Marked		Worse	
	Pr	Con	Pr	Con	Pr	Con	Pr	Con	Pr	Con
4–6	26	26	14	26	12	0	0	0	0	0
6–12	26	25	12	25	14	0	0	0	0	0
12–24	17	18	6	17	7	1	2	0	2	0
24–36	11	13	2	11	5	1	2	0	2	1

Pr: Prednisone group.
Con: Control group.

in the control group. The results of this study suggest that prednisone is beneficial up to six months and that further continuation of this drug does not elicit any improvement.

In summary: Twenty-six patients who did not improve in spite of intensive antimicrobial therapy were given a daily dose of 10 mg. of prednisone from four to nine months. Approximately half of these patients showed significant improvement and were discharged from the hospital with inactive disease. In the control group, only 2 patients had similar outcomes. With this dosage, no side reactions were noted in any of the patients in spite of prolonged therapy. These findings suggest that the addition of prednisone be considered for patients who fail to improve with conventional antimicrobial therapy. In the present study this adjunct therapy substantially reduced the number who might otherwise have become chronic patients.

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THE USE OF EGG-YOLK DIALYSATE FOR THE GROWTH OF MYCOBACTERIUM TUBERCULOSIS[1]

In 1902, Dorset[2] reported that the incorporation of eggs into medium enhanced the growth of tubercle bacilli. Egg yolk alone was found by Corper and Cohn[3] to support better growth of the organisms than the whole egg. Since these reports, many egg-containing media have been devised for the growth of *Mycobacterium tuberculosis*. Growth of tubercle bacilli occurs on these media, but it is slow and sometimes irregular depending upon the source of the eggs. A medium permitting quicker and more uniform growth of the organism would be advantageous. The present writers have found that the incorporation of egg-yolk dialysate into medium results in a preparation which provides more rapid and uniform growth of mycobacteria.

Fertile or nonfertile chicken eggs were obtained from the open flock of a local supplier, and were used either fresh or after a short incubation period of three to five days. The eggs were soaked several minutes in 70 per cent ethanol, and then were opened aseptically into a sterile Petri dish. The yolk material was removed with a sterile syringe. An equal volume of ethyl ether was added to 60 to 80 ml. of pooled yolk in a separatory funnel. The mixture was shaken for several minutes, and the ether layer withdrawn. This procedure was repeated several times until the yolk residue was almost devoid of color and ether-extractable materials. The residue was transferred to sterile cellophane dialysis tubing contained within a liter flask, and 500 ml. of sterile water, redistilled from glass, pH 5.0, were added to the flask. Dialysis was carried out for one week at 37°C. without changing the dialyzing medium. The dialysate was retained and the residue discarded. Great care was taken during the entire preparative procedure to assure complete asepsis because earlier experiments demonstrated that contaminated yolk contained no growth-promoting activity.

The test organisms employed were *M. tuberculosis*, strain H37Rv, and a nonphotochromogenic Mycobacterium organism, labeled strain 236, isolated from a human case of pulmonary disease. Inocula were harvested from Löwenstein-Jensen egg medium (BBL), and treated for a few minutes in a tissue homogenizer to break up the clumps. The suspension was washed twice in the medium of Proskauer and Beck,[4] and then diluted in the same medium so that the suspensions permitted only 90 per cent light transmission when measured in a Coleman Junior spectrophotometer at 660 mμ. The inoculum consisted of 0.2 ml. of this suspension.

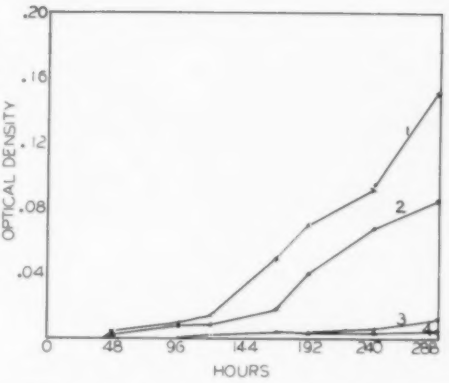

FIG. 1. Growth response of *Mycobacterium tuberculosis*, strain H37Rv, and nonphotochromogenic Mycobacterium, strain 236, to egg-yolk dialysate contained in Proskauer and Beck medium. Curve 1, strain 236 in dialysate plus medium; 2, H37Rv in dialysate plus medium; 3, H37Rv in medium alone; and 4, strain 236 in medium alone. The cultures were incubated at 37°C.

The results obtained when the two test organisms were inoculated into the asparagine-glycerol minimal medium of Proskauer and Beck,[4] containing 10 per cent of undiluted egg-yolk dialysate, are presented in figure 1. The turbidity of the liquid cultures was determined by measuring optical density in a Coleman Junior spectrophotometer. Before measuring the turbidity the cultures were thoroughly dispersed by agitation. Plate counts carried out on the suspensions during the experiment indicated that the increase in turbidity was the result of an increase in viable cell units. As may be seen from the figure, the two organisms demonstrated very little growth response in the Proskauer and Beck medium alone. However, when egg-yolk dialysate was included in the medium, a marked response occurred. Within five days both organisms showed signifi-

[1] This investigation was supported in part by research grant E-2365 from the Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[2] DORSET, M.: Am. Med., 1902, *3*, 555.

[3] CORPER, H. J., AND COHN, M. L.: Am. J. Hyg., 1933, *18*, 1.

[4] PROSKAUER, B., AND BECK, M.: Ztschr. Hyg. Infectionskrankh., 1894, *18*, 128.

TABLE 1

GROWTH RESPONSE OF M. TUBERCULOSIS H37Rv ON SOLID MEDIA

Medium	Growth Response at Day					
	5	7	18	21	28	35
Lowenstein Jensen	0	0	0	+	++	+++
American Trudeau Society	0	0	0	+	++	+++
Proskauer-Beck plus dialysate	0	±	++	+++	+++	+++
Proskauer-Beck alone	0	0	0	0	±	+

Amount of growth determined by number of colonies appearing on media surface and graded from 0 (no colonies) to ++++ (surface completely covered with growth). Cultures incubated at 37°C. in screw-cap tubes.

cant growth, and at twelve days the tubes were translucent, as shown by the optical density values. The nonphotochromogenic organism always evidenced greater growth than did tubercle bacilli.

Dilution of the dialysate resulted in decreased growth responses proportional to the extent of dilution. When the material was diluted beyond 1:1,000 the nonphotochromogenic organism did not grow any more than it did in Proskauer and Beck medium alone. The growth response of H37Rv was lost at dilutions of more than 1:100. Concentration of the dialysate resulted in greater growth stimulation. The activity of the dialysate in strain 236 was not lost by autoclaving or by exposure to extreme acid or alkaline pH. Autoclaving and exposure to alkali decreased the response of H37Rv to the dialysate. Neither the ether extract of yolks nor the residue left after dialysis demonstrated activity. As mentioned previously, no growth activity was elicited by dialysates prepared from contaminated yolk. Bacteriologic examination of contaminated yolk revealed that a variety of Gram-positive and -negative organisms, as well as yeasts, could be the responsible agents.

In addition to determining growth stimulation by the dialysate in liquid medium, experiments also have been carried out employing solid media. The results obtained by growth of the test organism, H37Rv, on the surface of 2 per cent agar slants of Proskauer and Beck medium containing 10 per cent of undiluted dialysate were compared with the results obtained with two conventional solid egg media designed for the growth of *M. tuberculosis*, Löwenstein-Jensen medium (BBL) and American Trudeau Society medium (BBL). The data from these experiments are presented in table 1. All media were inoculated from the same bacterial suspension. It is readily apparent that egg-yolk dialysate stimulated the growth of H37Rv on agar medium. Microscopic examination of the growth appearing on all of the media demonstrated acid-fast bacilli morphologically identical to mycobacteria.

The experiments presented in figure 1 and table 1 have been repeated many times, employing different samples of egg-yolk dialysate. In all cases the results were similar to those already described. Identical responses were obtained employing dialysate prepared from either fertile or nonfertile eggs. No difference in growth-promoting activity was observed between fresh eggs and eggs that had been incubated for as long as five days. As a matter of fact, the latter eggs were those that had been rejected by a virus laboratory as unsuitable for their experiments.

Hirsch[5] and Yamane[6] have reported the extraction of substances from egg yolk which would stimulate the growth of mycobacteria. Both of the substances were soluble in ether, and Hirsch[5] identified his substance as cholesterol. The stimulatory material present in egg-yolk dialysate was not readily soluble in ether, and chemical tests on concentrated dialysates for the presence of cholesterol were negative. Thus the substance does not appear to be the same as those materials extracted by Hirsch[5] and Yamane.[6] Experiments carried out employing oleic acid established that no growth stimulation occurred in Proskauer and Beck medium unless both the acid and albumin were added. The latter substance was not present in the dialysate. Additional investigations demonstrated that casein hydrolysate, yeast extract, or mineral salt solutions would not stimulate growth of mycobacteria in Proskauer and Beck medium. The relationship of the substance present in egg-yolk dialysate to the polysaccharide material isolated from tender coconuts, and reported to

[5] HIRSCH, J. G.: Am. Rev. Tubere., 1954, *70*, 977.

[6] YAMANE, I.: J. Bact., 1957, *73*, 334. The writers have attempted to repeat this procedure, but have been unable to obtain any crystalline growth-promoting substance.

promote the growth of *M. tuberculosis* H37Rv,[7] is under investigation.

In summary: Data have been presented which indicate that egg-yolk dialysate will promote the growth of mycobacteria. Yolk material was obtained aseptically, extracted with ethyl ether, and the residue was dialyzed against distilled water. Turbidimetric measurements demonstrated that the dialysate when incorporated into Proskauer and Beck medium markedly stimulated growth of *Mycobacterium tuberculosis*, strain H37Rv. Growth on agar media of similar composition was more rapid than growth on conventional egg media.

JAMES O. NORMAN[8]
ROBERT P. WILLIAMS
Department of Microbiology
Baylor University College of Medicine
Houston, Texas

September 23, 1959

[7] RAMAKRISHNAN, T., INDIRA, M., AND SIRSI, M.: Nature, 1957, *179*, 1356.

[8] The writers are indebted to Mrs. Francis C. Whitcomb, Bacteriologist in Charge, Division of Microbiology; and to Dr. Bela Halpert, Chief of Laboratory Services, Veterans Hospital, Houston, Texas, for their cooperation in providing eggs and laboratory space during the early phases of the work.

Correspondence

UNCLASSIFIED MYCOBACTERIA FROM HUMAN SOURCES

To the Editor of The American Review of Respiratory Diseases:

In correspondence which recently appeared in the REVIEW, Runyon[1] raised several questions regarding reports which Burrows and Barclay[2] and I[3] had published. In this communication I wish to call attention to some data which will not only corroborate the findings of Burrows and Barclay, but also may throw some additional light on the problem of unclassified mycobacteria.

In an effort to determine the frequency of chromogenic acid-fast bacilli in patients free from active tuberculosis and who had never received antituberculosis chemotherapy, Burrows and Barclay cultured the basal gastric aspirate obtained in the course of routine gastric secretory tests. On the basis of their observations, it was concluded: "The fact that chromogenic mycobacteria could be cultured from the gastric juice of only one of 400 nontuberculous patients suggests that these organisms are infrequent incidental saprophytes in the population as a whole. The frequent isolation of such organisms in tuberculosis sanatoriums would seem related in some way to tuberculous infection or to the chemotherapy thereof."

Runyon stated that the finding of only one organism in this number of patients appeared to be too low, and that one might anticipate a higher proportion if the study were continued over a longer period of time, through several seasons; or that these investigators would have found the same low incidence in specimens from tuberculous or recently tuberculous patients.

From December, 1946 through December, 1948, at the Veterans Administration hospital in Walla Walla, Washington, we[4, 5] conducted a similar study to that of Burrows and Barclay using essentially similar methods, but included for comparison tuberculous patients who had received or were receiving streptomycin therapy. Two hundred and three nontuberculous and 405 tuberculous patients were studied. Only two (0.9 per cent) chromogenic mycobacteria were isolated from the former group of patients, whereas seventy-seven (19.0 per cent) were recovered from the latter group.

It was chiefly because of these findings that studies were initiated in an effort to determine whether exposure *in vitro* of human tubercle bacilli to any of the antituberculosis drugs would produce chromogenic ("atypical") variants; and if so, in what ways, if any, the variants differed from the parent organisms.

Preliminary observations made over a twelve-year period have recently been reported.[3] By the procedures used, under the conditions described, it was concluded that streptomycin- and isoniazid-resistant, chromogenic, atypical, nonacid-fast variant strains could be produced from the parent H37Rv organism. On the basis of preliminary cultural, pathologic, allergenic, and antimicrobial susceptibility studies, these variants appear genetically related to the parent organism. Similar results have been reported by Di Marco and his associates[6] and Xalabarder.[7, 8]

It is Runyon's opinion that the variant strains which I have described are not comparable with the unclassified ("atypical") mycobacteria, and that it may legitimately be questioned whether these strains are mycobacteria since they are not acid fast. In the light of our limited knowledge of the unclassified mycobacteria, and on the basis of the inadequacy of any presently available methods of classifying such organisms, it would appear premature to draw such definite conclusions. There are numerous reports of aberrant strains of mycobacteria associated with disease processes in man which do not fit clearly into any method of classification.

The influence of antimicrobial drugs and other agents on bacterial variation has already been discussed in detail.[3] If so many bacterial species, including some mycobacteria, can be altered so profoundly in morphology, staining, metabolic activity, virulence, and other characteristics, why then should it appear so remarkable if human strains of tubercle bacilli can also be altered by such influences?

Perhaps to some, a more acceptable hypothesis is that drug influence in the case of tuberculosis

[1] RUNYON, E.: Am. Rev. Resp. Dis., 1959, *80*, 277.

[2] BURROWS, B., AND BARCLAY, W. R.: Am. Rev. Tubere., 1959, *79*, 543.

[3] TARSHIS, M. S.: Am. Rev. Tubere., 1958, *78*, 921.

[4] SCHIFF, J., AND TARSHIS, M. S.: Northwest Med., 1950, *49*, 451.

[5] TARSHIS, M. S.: Unpublished data.

[6] DI MARCO, A., *et al.*: Gior. microbiol., 1956, *1*, 266.

[7] XALABARDER, C.: Ciba Symposium, 1955, *3*, 19.

[8] XALABARDER, C.: Personal communication.

is more of an indirect phenomenon, resulting in the production of aberrant strains of tubercle bacilli as a consequence of *in vivo* physico-chemical mechanisms which come into play following clinical improvement of patients. Some of the intrinsic mechanisms which operate not only in altering the course of microbial infection and disease, but also in modifying the characteristics of the organisms, were discussed by Dubos.[9] Alterations in biochemical status follow clinical improvement whether improvement has been achieved by drugs or other forms of therapy. That tubercle bacilli may be altered *in vivo* has been suggested in the reports of Pinner,[10] Wenkle and associates,[11] Schiff and Tarshis,[4] and Keltz and his co-workers,[12] all of whom noted that, when chromogens were isolated from patients, many of the patients were in a clinically improved phase of their disease. Accordingly, this may also explain why chromogenic acid-fast bacilli were isolated from patients before the advent of chemotherapy, but apparently never to the same degree as are being isolated today. However, some workers believe that such an increase is more apparent than real on the basis that this is only because such organisms are being examined more closely and critically than formerly, and that the use of drug therapy has only reduced the number of positive cultures for tubercle bacilli, thus accentuating the finding of the more highly resistant chromogenic mycobacteria. Others feel that these types of organisms are not and never were tubercle bacilli. Rather, they probably represent unrecognized and unclassified members of the genus Mycobacterium, and that such strains may or may not be pathogenic for man. These views may be true, but do they necessarily explain the origin of all the different types of unclassified mycobacteria?

Although the variant strains which I have described were originally essentially nonacid fast, some substrains have now become either partially or completely acid fast as a result of long subculture in media containing an extract of the parent organism, or by subculture in Proskauer-Beck liquid medium. Shifts back and forth from acid-fastness, partial acid-fastness, or nonacid-fastness have also been noted from time to time, depending on the environmental conditions. The fact that an organism does not stain acid fast at any observation point does not necessarily rule it out as a Mycobacterium.

Although the subject of mycobacterial nonacid-fastness is complex and controversial, it is well known that the integrity of the cell structure is essential for the maintenance of acid-fastness. Environmental influences, either mechanical or chemical, may alter this property. For example, the effect of isoniazid, cycloserine, and α-ethyl-thioisonicotinamide on morphology and loss of acid-fastness of the H37Rv strain of *M. tuberculosis* and other mycobacteria was reported by Middlebrook,[13] Schaefer,[14] Koch-Weser and associates,[15] Barclay and Russe,[16] and Dunbar.[17] Numerous studies have been reported in which nonacid-fast forms are thought to represent a stage in an evolutionary life cycle of the mycobacteria. The fact that antimicrobial agents are able to alter the morphology, staining, and other properties of mycobacteria may possibly explain why chromogens are not found as frequently in the sputum or resected lung lesions in drug-treated patients, since such altered strains would no longer be recognizable by the usual methods of detection. This view is also supported by the fact that certain pathological specimens which do not show tubercle bacilli on direct examination may indeed produce tuberculosis in the guinea pig.[7]

In the light of recent contributions in the field of bacterial genetics, and what is known regarding the influence of antimicrobial drugs on bacterial variation, is it not time perhaps to take a new, a longer, and a more critical look at what antituberculosis chemotherapy might possibly be doing to the tubercle bacillus? The dogma of the monomorphism of this organism is no longer tenable. The classical descriptive morphologic characteristics of *M. tuberculosis* have been seriously questioned time and again by various investigators. For example, Xalabarder[18] has demonstrated by means of comprehensive electron microscopic studies that under certain environmental conditions tubercle bacilli undergo a

[9] Dubos, R. J.: Biochemical Determinants of Microbial Diseases, ed. 1, Harvard University Press, Cambridge, Massachusetts, 1954.

[10] Pinner, M.: Pulmonary Tuberculosis in the Adult, ed. 1, Charles C Thomas, Springfield, Illinois, 1946.

[11] Wenkle, W. C., *et al.*: Am. Rev. Tuberc., 1948, *57*, 385.

[12] Keltz, H., *et al.*: Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1957.

[13] Middlebrook, G.: Am. Rev. Tuberc., 1952, *65*, 765.

[14] Schaefer, W.: Am. Rev. Tuberc., 1954, *69*, 125.

[15] Koch-Weser, D., *et al.*: Am. Rev. Tuberc., 1955, *71*, 556.

[16] Barclay, W. R., and Russe, H.: Am. Rev. Tuberc., 1955, *72*, 236.

[17] Dunbar, J. M.: Ann. Inst. Pasteur, 1957, *92*, 451.

[18] Xalabarder, C.: Pub. Inst. Antituberculoso Francisco Moragas, Barcelona, 1954.

AN ANALYSIS OF THE TYPES AND DISTRIBUTION OF MYCOBACTERIA FOUND IN 65,949 UNCONTAMINATED* CULTURE SPECIMENS SUBMITTED DURING 1958 TO THE BUREAUS OF TUBERCULOSIS AND LABORATORIES, DEPARTMENT OF HEALTH, CITY OF NEW YORK

Type of Specimen Submitted	Total†		Positive for *M. Tuberculosis*‡		Positive For Unclassified Mycobacteria§								Ratio of Number of Unclassified Acid-fast Bacilli to Total Number of Acid-fast Bacilli	
					Scotochromogenic		Photochromogenic		Nonchromogenic		Total			
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	65,949	100.0	4,201	6.4	309	0.47	12	0.02	87	0.13	408	0.62	408/4609	8.9
Sputum	53,355	100.0	3,768	7.0	170	0.32	12	0.02	77	0.14	259	0.48	259/4027	6.4
Gastric aspirate	4,710	100.0	244	5.2	73	1.55	0	—	5	0.11	78	1.66	78/322	24.2
Laryngeal swab	7,150	100.0	131	1.8	63	0.89	0	—	4	0.06	67	0.94	67/198	33.8
Urine	601	100.0	17	2.8	2	0.33	0	—	1	0.17	3	0.50	3/20	15.0
Miscellaneous‖	133	100.0	41	30.8	1	0.75	0	—	0	—	1	0.75	1/42	2.4

* 2,472 specimens (3.6% of total) were contaminated with organisms other than mycobacteria.

† In 18 instances, the same type of unclassified Mycobacterium was isolated from more than one specimen submitted by a single patient.

‡ 8 sputum cultures positive for *M. tuberculosis* also grew out scotochromogenic mycobacteria and 1 sputum culture grew out both *M. tuberculosis* and a nonchromogenic unclassified Mycobacterium.

§ In 3 instances (1 sputum, 1 gastric, and 1 laryngeal swab culture) two different types of unclassified mycobacteria were isolated from a single specimen.

‖ Chest fluid, cerebrospinal fluid, purulent secretions, synovial fluid.

complex cycle, exhibiting spores, flagella, motility, and a reproductive cycle quite different from that of simple binary fission. Even their acid-fastness is transient and unstable during their life-history. That mycobacteria as well as other bacterial species might possibly exist in more than one form, depending on the environmental conditions, has also recently been discussed by McDermott.[19]

In view of this newer knowledge, does it appear untenable that some strains of unclassified mycobacteria at least could probably have been derived from tubercle bacilli as the result of drug therapy?

MAURICE S. TARSHIS
Medical Research Laboratory
Veterans Administration Hospital
Alexandria, Louisiana

September 1, 1959

UNCLASSIFIED MYCOBACTERIA

To the Editor of the American Review of Respiratory Diseases:

Attached is a table showing the results of the recently conducted study of unclassified mycobacteria isolated from culture specimens submitted to the Bureau of Laboratories during 1958 for the detection of tubercle bacilli. Almost all of the specimens were from ambulatory patients.

The figures given in the table are self-explanatory. It was the impression of this observer that in all instances the unclassified mycobacteria isolated were contaminants and not responsible for the development of any pulmonary disease. This conclusion is based on a review of the charts and roentgenograms of all patients with more than one culture positive for unclassified mycobacteria, and in all instances in which photochromogenic or nonchromogenic organisms were found.

It should be emphasized that the population from which this material was derived is a clinic population. The situation may be otherwise among a hospitalized population. In this connection, starting on January 1, 1960, a study is contemplated to determine the presence and significance of unclassified mycobacteria in patients, both from the clinic and the hospital, considered to have newly diagnosed pulmonary tuberculosis. All that can be said at present is that, in New York City, unclassified mycobacteria are found frequently in sputum specimens of chest clinic patients, but that this group of organisms has not

[19] McDERMOTT, W.: The Dyer Lecture, Pub. Health Rep., 1959, 74, 485.

been found to be a significant cause of pulmonary disease in this population.

Of some interest is the fact that a higher percentage of unclassified mycobacteria were found in laryngeal swab and gastric aspiration specimens than in sputum specimens. This finding confirms similar observations reported from other clinics.

AARON D. CHAVES[1]
Supervisor of Clinics, Bureau of Tuberculosis
Department of Health
New York, New York

October 2, 1959

PS. In this connection, I enclose a letter from Dr. Ernest H. Runyon. One hundred and fourteen sample specimens of unclassified mycobacteria isolated in the study in New York City were sent to Dr. Runyon for classification and we learned of the results only recently, as you can see. His comments are of interest inasmuch as they show the difficulty that a routine laboratory such as ours has in accurately identifying not only the various groups of unclassified mycobacteria but also the various "atypical" human strains of tubercle bacilli. I might say that we checked the clinical records of the three patients whose cultures were originally designated "atypical" by us, but as true tubercle bacilli by Dr. Runyon, and found that in all three instances the patients had unequivocal pulmonary tuberculosis with readily recognizable tubercle bacilli identified from other specimens. A.D.C.

[1] The laboratory work on the study was done by Mrs. G. Dangler under the direction of Dr. Daniel Widelock.

Dear Dr. Chaves:

I have just returned from Europe and find your inquiry concerning the identification of cultures sent to us from your clinic patients. This has been an interesting study. Many of your strains have been different from those we have obtained elsewhere.

Enclosed are the cards with our identification number and classification entered in red ink. As you will see, most of the strains received have been scotochromogens (Group II) or nonphotochromogens (Group III). Many of your Group III strains, however, are distinct in one way or another from the Battey bacillus. Other notable findings are:

Three cultures were designated "atypical" by you, but we identify them as true tubercle bacilli ("TB").

We have not run animal tests, but these cultures produce niacin and have cultural characteristics of *M. tuberculosis*.

Only one photochromogen was identified. This was not one of those recognized by you. Indeed none of the nine strains designated on your cards as "Photo" were photochromogens according to our testing and definitions. The absence of Group I photochromogens is a striking feature of your series.

ERNEST H. RUNYON
Microbiology Research
Veterans Administration Hospital
Fort Douglas, Salt Lake City 13, Utah

November 6, 1959

Books

TUBERKULOSE-JAHRBUCH 1957. Edited by Rolf Griesbach. Pp. 291. Springer-Verlag, Berlin, Gottingen, Heidelberg, Germany, 1959.

The book under review represents an official publication issued by the West German Central Committee for the fight against tuberculosis, which reports on the tuberculosis situation in West Germany for the year 1957. Morbidity and death statistics, reports on the incidence and results of BCG vaccination, and discussions about the public health, medicolegal and social aspects of the disease are to be found in this volume and permit the interested reader to acquaint himself quickly and exhaustively with the pertinent facts. In West Germany, as in other countries, mortality from tuberculosis has dropped at a much faster rate than morbidity, while the number of chronically ill has remained fairly stable, viz., 20.5 per 10,000. At present, 6–7 per cent of patients with open tuberculosis continue to die from the disease annually. The formidable public health aspects are emphasized by the fact that more than 30 per cent of patients with open cases do not possess rooms of their own, and about 1 per cent share their beds with another person. Although new infections in the age group from one to ten years have decreased considerably, 3–4 per cent of all males more than thirty years of age continue to have active or inactive tuberculosis, and the public health authorities keep 650,000 inactive cases under observation. Great efforts are made to provide every citizen with an annual roentgenographic examination of the chest; however, on the basis of available statistics, it is assumed that only 35 per cent of all new cases are detected. The annual cost to maintain a patient runs at present about 3160.-DM ($760) per year, which includes neither drugs nor surgical procedures.

To alleviate the social suffering connected with the disease, an increasing number of public and private employes are protected by law against losing their jobs should they become ill. In order to provide the university student afflicted with tuberculosis with as much education as his status permits, the West German government has opened the St. Blasien, a sanitarium organized especially for this group, where opportunities to attend lectures and seminars are offered.

Prevention continues to be a formidable problem. BCG vaccination, which is widely practiced in central Germany, has not become popular with the general public in West Germany, although all competent observers agree as to its positive value.

These few examples may suffice to inform the reader about the scope of this statistical report, which should be studied by all who wish to inform themselves about the magnitude of the tuberculosis problem in Europe. Each chapter of the publication is provided with a short summary in English which is sufficiently comprehensive to make understanding possible to the reader who is unfamiliar with German. An appendix reviews the most important meetings held in Europe concerning matters connected with the pathology, treatment, and prevention of tuberculosis during the year 1957.

DIETHELM BOEHME
New York, New York

ANNUAL REVIEW OF MEDICINE. Volume 10. Edited by David A. Rytand and William P. Creger. Pp. 448, Annual Reviews, Inc., Palo Alto, California, 1959. Price, $7.00.

This volume, like its predecessors in this series, is a valuable collection of literature reviews on selected topics in medicine. The individual articles are for the most part clear and authoritative. Of particular interest are the review on salmonellosis by Drs. Bennett and Hook, Dr. Dock's concise but comprehensive paper on atherosclerosis, and the article of Drs. Clark and Sutow on tumor chemotherapy. Dr. Finch has contributed an excellent review of recent work on blood transfusion.

The annotated list of reviews in medicine, a feature of previous volumes, has again, unfortunately, been omitted.

This volume serves as an excellent entry to the literature in selected fields of medicine, as well as offering, in fairly compact fashion, some insight into an enormous amount of clinical and human biologic research.

JEREMIAH A. BARONDESS
New York, New York

BOOKS RECEIVED

METABOLIC CARE OF THE SURGICAL PATIENT. By Francis D. Moore. Pp. 1011, W. B. Saunders Company, Philadelphia, London, 1959. Price, $20.00.

1959–60 YEAR BOOK OF GENERAL SURGERY. Edited by Michael E. De Bakey. Pp. 639, Year Book Publishers, Inc., Chicago, 1959. Price, $8.00.

HANDBOOK OF CIRCULATION. Edited by Dorothy S. Dittmer and Rudolph M. Grebe. Pp. 393, W. B. Saunders Company, Philadelphia, London, 1959. Price, $7.50.

MAN IN A COLD ENVIRONMENT. Physiological and Pathological Effects of Exposure to Low Temperatures. By Alan C. Burton and Otto G. Edholm. Pp. 273, Edward Arnold Ltd., London (The Williams & Wilkins Co., Baltimore, exclusive U.S. agents), 1955. Price, $6.75.

ANTIBIOTICS MONOGRAPHS NO. 12: ANTIBIOTIC THERAPY FOR STAPHYLOCOCCAL DISEASES. Edited by Henry Welch and Maxwell Finland. Pp. 208, Medical Encyclopedia, Inc., New York, 1959. Price, $4.50.

TUBERCULOSE PULMONAIRE. ROLE DES GANGLIONS LYMPHATIQUES. By Ph. Schwartz. Pp. 230, Masson et Cie., Paris, 1959. Price, 3,000 fr.

A GUIDE TO ANTIBIOTIC THERAPY. By Henry Welch. Pp. 69, Medical Encyclopedia, Inc., New York, 1959. Price, $3.00.

THE ACUTE AND CHRONIC PEPTIC LESIONS OF THE STOMACH AND THE DUODENUM. Their Frequency, Mutual Relation and Correlation with Other Diseases. By I. S. Levij. Pp. 104, Uitgeverij Excelsior, The Netherlands, 1959.

American Trudeau Society

Medical Section of the National Tuberculosis Association

PRELIMINARY PROGRAM, MEDICAL SESSIONS, AMERICAN TRUDEAU SOCIETY ANNUAL MEETING

MONDAY, MAY 16

10:45 a.m.—12:15 p.m.

1A The Differentiation of Mycobacteria

Changes in the Tuberculin Pattern in Medical Students between 1930 and 1960—DAVID T. SMITH, M.D., AND WILLIAM W. JOHNSTON, M.D., Durham, N. C.

Discussion

A Study of Reactions to Various Anonymous Mycobacterial Antigens in Dallas County Orphans—HAL J. DEWLETT, M.D., L. LEFKOWITZ, R. FULAYFIL, LYNN C. CHRISTIANSON, M.D., AND JOHN S. CHAPMAN, M.D., Dallas, Texas

Discussion

Epidemiologic Interpretation of Comparative Tuberculin Testing—H. M. VANDIVIERE, M.A., M. R. VANDIVIERE, M.A., AND H. S. WILLIS, M.D., Chapel Hill, N. C.

Discussion

Antigenic Response to Mycobacterial Infection—CARROLL E. PALMER, M.D., LYDIA B. EDWARDS, M.D., LEWIS F. AFFRONTI, PH.D., LOUISE HOPWOOD, M.A., AND PHYLLIS Q. EDWARDS, M.D., Washington, D. C.

Discussion

Intracellular Growth of Tubercle Bacilli and Other Mycobacteria in the J-111 Cell Line of Leukemic Monocytes—EDWIN A. BROSBE, PH.D., PAUL T. SUGIHARA, B.A., AND C. RICHARD SMITH, M.D., Long Beach, Calif.

Discussion

10:45 a.m.—12:15 p.m.

1B Emphysema

The Elastin and Collagen Content of Normal and Emphysematous Human Lungs—GEORGE W. WRIGHT, M.D., JEROME KLEINERMAN, M.D., AND ELINOR M. ZORN, M.S., Cleveland, Ohio

Discussion

Prognostic Factors in Pulmonary Emphysema—GEORGE BOWER, M.D., Denver, Colo.

Discussion

The Incidence of Obstructive Emphysema in Patients with Tuberculosis—W. Y. HALLETT, M.D., AND C. J. MARTIN, M.D., Seattle, Wash.

Discussion

The Incidence of Diffuse Airway Obstruction in an Industrial Population—GEOFFREY L. BRINKMAN, M.D., AND E. OSBORNE COATES, JR., M.D., Detroit, Mich.

Discussion

Comparison of Lung Function Tests Based on Maximal Expiratory Effort—JOHN R. GOLDSMITH, M.D., Berkeley, Calif.

Discussion

12:30 p.m.—1:45 p.m.

Sandwich Seminar I*

Practical Pulmonary Function Testing—JOHN KIERAN, M.D., Oakland, Calif., *Moderator*

Panelists: KENNETH T. BIRD, M.D., Boston, Mass., and FREDERICK C. WARRING, JR., M.D., Shelton, Conn.

Sandwich Seminar II*

Compulsory Isolation of the Recalcitrant Patient—JOSEPH B. STOCKLEN, M.D., Cleveland, Ohio, *Moderator*

Panelists: EDWARD KUPKA, M.D., Berkeley, Calif., and CEDRIC NORTHROP, M.D., Seattle, Wash.

Sandwich Seminar III*

Treatment of the Chemotherapy Failure—PAUL T. CHAPMAN, M.D., Detroit, Mich., *Moderator*

* Each seminar will be limited to 35 physicians in order to allow for informal discussion with the experts. Tickets must be purchased in advance from the American Trudeau Society.

Panelists: MEYER LICHTENSTEIN, M.D., Chicago, Ill., and PATRICK STOREY, M.D., Baltimore, Md.

Sandwich Seminar IV*

Air Pollution and Pulmonary Diseases—HERMAN GIERSON, M.D., Los Angeles, Calif., *Moderator*

Panelists to be announced

2:00 p.m.—4:00 p.m.

2A Clinical Chemotherapy

Coalminers' Pneumoconio-tuberculosis—Medical and Surgical Treatment—CHARLES GERNEZ-RIEUX, M.D., C. VOISIN, AND P. FOURNIER, Lille, France

Discussion

Chemotherapy of Pulmonary Photochromogenic Mycobacterial Infections in Man—LYNN C. CHRISTIANSON, M.D., PAUL L. RICHBURG, M.D., AND HAL J. DEWLETT, M.D., Dallas, Texas

Discussion

The Treatment of Tuberculosis of the Spine in Adults without Surgical Spine Fusion—BARRY FRIEDMAN, M.D., Cleveland Heights, Ohio

Discussion

The Management of Patients Excreting Acid-fast Organisms Resistant to Both Streptomycin and Isoniazid—IRVING KASS, M.D., JAAKKO HAAPANEN, M.D., AND W. F. RUSSELL, JR., M.D., Denver, Colo.

Discussion

Effects of Withdrawal of Isoniazid in Chronic, Stable, Treatment-Failure Cases with Drug-Resistant Tubercle Bacilli—EMANUEL WOLINSKY, M.D., AND VISHWA N. KAPUR, Cleveland, Ohio

Discussion

Streptomycin 0.5 Gm. Daily with Isoniazid and Para-aminosalicylic Acid Used as a Retreatment Regimen in Pulmonary Tuberculosis—WILLIAM S. SCHWARTZ, M.D., AND R. E. MOYER, M.D., Oteen, N. C.

Discussion

An Evaluation of Chemotherapy in Pulmonary Tuberculosis—High Doses of INH plus PAS versus Low Doses of INH plus PAS—LT. COL. STEPHEN J. BERTE, MC (USA), AND COL. WILLIAM G. DUNNINGTON, MC (USA), Phoenixville, Pa.

Discussion

2:00 p.m.—4:00 p.m.

2B Pulmonary Physiology

Hemodynamics of the Pulmonary Microcirculation—JOHN W. IRWIN, M.D., WALTER S. BURRAGE, M.D., AND FRANCIS L. WEILLE, M.D., Boston, Mass.

Discussion

The Effect of Bronchospasm on Pulmonary Airway (Tracheal, Bronchial, and Alveolar) and Circulatory Pressures—An Experimental Study—WATTS R. WEBB, M.D., LEONARD W. FABIAN, M.D., G. D. CAMPBELL, AND JOSEF R. SMITH, M.D., Jackson, Miss.

Discussion

Measurement of Gas Trapped in the Lung during Changing Airway Resistance—FRANK W. LOVEJOY, JR., M.D., HERBERT CONSTANTINE, M.D., JOSEPH FLATLEY, M.D., LUCIAN DAUTREBANDE, M.D., AND NOLAN L. KALTREIDER, M.D., Rochester, N. Y.

Discussion

Application of Gas Chromatography to Determinations of Pulmonary Blood Flow and Diffusing Capacity—W. HAROLD LAWSON, JR., M.D., AND ROBERT L. JOHNSON, JR., M.D., Dallas, Texas

Discussion

Ventilation-Perfusion Ratio Determination Using a Gas Partitioner—H. G. BOREN, M.D., AND F. L. KRACKE, Houston, Texas

Discussion

A Simple and Rapid Method of Determining Pulmonary Diffusing Capacity by the Carbon Monoxide Steady State Method—Stanford K. Sweany, M.D., Chicago, Ill.

Discussion

An Appraisal of the Breath-holding Method for Measuring DC in Patients with Nonuniformity of Alveolar Gas; and a Simplification of the Measurement of Alveolar Volume—NOLA MAE MOORE, M.D., THEODORE C. SMITH, M.D., ALICE L. CASTILLO, B.S., AND JOHN RANKIN, M.D., Madison, Wisc.

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* Each seminar will be limited to 35 physicians in order to allow for informal discussion with the experts. Tickets must be purchased in advance from the American Trudeau Society.

Discussion

Ventilation, Deadspace, and Hemoglobin Level as Modifiers of the Steady State Method for Diffusing Capacity for Carbon Monoxide—KAYE H. KILBURN, M.D., Denver, Colo.

Discussion

4:30 p.m.—5:30 p.m.

3A Newer Viral Respiratory Diseases

Moderator to be announced

Panelists to be announced

4:30 p.m.—5:30 p.m.

3B Steroids in Nontuberculous Pulmonary Diseases

Moderator to be announced

Panelists to be announced

4:30 p.m.—5:30 p.m.

Demonstration I

The Evaluation of Pulmonary Function in Chronic Respiratory Disease—HURLEY L. MOTLEY, M.D., University of Southern California Cardiorespiratory Laboratory. Limited to 20 physicians. Reservations must be obtained in advance from the American Trudeau Society.

Demonstration II

Pulmonary Evaluation of the Postoperative Patient—RONALD N. SELVESTER, M.D., C. JOAN COGGIN, M.D., AND ELLSWORTH E. WAREHAM, M.D., White Memorial Hospital of the College of Medical Evangelists. Limited to 30 physicians. Reservations must be secured in advance from the American Trudeau Society.

Demonstration III

The Diagnosis and Management of Acute Respiratory Failure—HERMAN W. GIERSON, M.D., GINO IOVINE, M.D., HUGH GAFFNEY, M.D., AND JACK HACKNEY, M.D., Los Angeles County General Hospital. Limited to 20 physicians. Reservations must be secured in advance from the American Trudeau Society.

4:30 p.m.—5:30 p.m.

Case Conference

Moderator to be announced

TUESDAY, MAY 17

7:15 a.m.—8:45 a.m.

California Trudeau Society Breakfast

Program to be announced

9:00 a.m.—10:45 a.m.

4A Pulmonary Physiology

Pathophysiologic Features of Pulmonary Alveolar Proteinosis with Notes on Differential Diagnosis—A Report of Six Cases—EDWARD A. GAENSLER, M.D., ASHER MARKS, M.D., AND EUGENE D. ROBIN, M.D., Boston, Mass.

Discussion

Unilateral Hypoventilation during Temporary Ipsilateral Pulmonary Artery Occlusion in Man—E. W. SWENSON, M.D., T. N. FINLEY, M.D., S. V. GUZMAN, M.D. AND J. W. SEVERINGHAUS, M.D., San Francisco, Calif.

Discussion

The Effect of Hyperventilation and of Bronchodilator Drugs on Alveolar Ventilation in Patients with Chronic Obstructive Pulmonary Emphysema—AARON A. COHEN, M.D., AND ALLAN HEMINGWAY, PH.D., San Fernando, Calif.

Discussion

Pulmonary Diffusing Capacity in Resting Subjects with Normal and Increased Pulmonary Blood Flow—GEORGE N. BEDELL, M.D., Iowa City, Iowa

Discussion

The Effects of Thoracic Surgery on the Pulmonary Diffusing Capacity—FRANCES R. DIETIKER, B.A., AND BENJAMIN BURROWS, M.D., Hinsdale, Ill.

Discussion

The Relationships of Hypercapnia to Thyroid Function—MYRON STEIN, M.D., DAVID SINGER, M.D., AND MILTON W. HAMOLSKY, M.D., Boston, Mass.

Discussion

The Effect of Isoproterenol on the Heart and Pulmonary Circulation in Patients with Chronic Pulmonary Emphysema—JOHN F. WILLIAMS, M.D. DOUGLAS H. WHITE, M.D., AND ROY H. BENNKE, M.D., Indianapolis, Ind.

Discussion

9:00 a.m.—10:45 a.m.

4B Chemotherapy, Experimental

A Simple Test to Distinguish "Rapid" and "Slow" Inactivators of Isoniazid—H. WILLIAM HARRIS, M.D., RALPH A. KNIGHT, PH.D., DORIAN R. FABER, B.S., AND MERLE J. SELIN, B.S., Salt Lake City, Utah

Discussion

Effect of Cortisone plus Antituberculosis Agents on Tuberculous Pneumonia in Guinea Pigs—Preliminary Report—ALFRED G. KARLSON, D.V.M., AND DAVID T. CARR, M.D. Rochester, Minn.

Discussion

The Chemotherapy of Experimental Pulmonary Tuberculosis—Correlation between Survival of Rabbits and the in Vitro *Demonstration of Viable Tubercle Bacilli*—SIDNEY BERNSTEIN, M.S., AND NICHOLAS D. D'ESOPO, M.D., West Haven, Conn.

Discussion

Correlation of Drug Susceptibility and Metabolic Activity of Various Strains of Mycobacteria—D. KOCH-WESER, M.D., AND R. P. PALMER, A.B., Cleveland, Ohio

Discussion

A Rapid Method of Testing the Susceptibility of Mycobacteria to Antituberculosis Drugs—C. O. ROGERS, A.B., I. G. MELVIN, M.S., AND H. S. WILLIS, M.D., Chapel Hill, N. C.

Discussion

A Rapid Drug Sensitivity Test for Acid-fast Bacilli—G. P. KUBICA, PH.D., Chamblee, Ga.

Discussion

9:00 a.m.–10:45 a.m.

4C Fungous Diseases

Report on Amphotericin B—W. D. SUTLIFF, M.D., Memphis, Tenn.

Discussion

Observations on Experimental Histoplasmosis in Dogs—JOHN J. PROCKNOW, M.D., EVELYN SAXON, B.S., AND C. GEORGE RAY, B.S., Chicago, Ill.

Discussion

The Serious Prognosis of Untreated Histoplasmosis—FRED E. TOSH, M.D., IRENE L. DOTO, M.A., AND MICHAEL L. FURCOLOW, M.D., Kansas City, Kans.

Discussion

High Incidence of Histoplasmosis in Sanatoriums in the United States—MICHAEL L. FURCOLOW, M.D., JOSEPH H. SCHUBERT, PH.D., AND FRED E. TOSH, M.D., Kansas City, Kans.

Discussion

Nocardiosis—An Analysis of Nine Cases—JOHN F. MURRAY, M.D., SIDNEY M. FINEGOLD, M.D., SEYMOUR FROMAN, PH.D., AND DRAKE W. WILL, M.D., Los Angeles, Calif.

Discussion

11:00 a.m.—12:00 Noon

The Amberson Lecture—*Aspects of Contemporary Research in Immunology*—SIDNEY RAFFEL

12:15 p.m.—1:45 p.m.

American Trudeau Society Luncheon

Program to be announced

2:00 p.m.—4:00 p.m.

5A Surgery

Early Resection—RICHARD F. KIEFFER, JR., M.D., Baltimore, Md.

Discussion

The Effects of Extraperiosteal Thoracoplasty with Paraffin Plombage on Pulmonary Function—GORDON L. SNIDER, M.D., ALLAN R. SHAW, AND WILLIAM M. LEES, M.D., Chicago, Ill.

Discussion

Cavernostomy—A Salvage Procedure for Giant Tuberculous Cavities—J. N. BRIGGS, M.D., AND R. R. TORREY, M.D., Encino, Calif.

Discussion

Pulmonary Resection for Emphysematous Bullae—David M. Sensenig, M.D., and Theodore Rodman, M.D., Philadelphia, Pa.

Discussion

The Surgical Management of Large Pulmonary Blebs and Bullae—Harold C. Spear, M.D., Dewitt C. Daughtry, M.D., John G. Chesney, M.D., and Asher Marks, M.D., Miami, Fla.

Discussion

The Surgical Treatment of Pectus Excavatum Utilizing an Adhesive Hemicast—Colin S. Dafoe, M.D., and Colin A. Ross, M.D., Edmonton, Alberta, Canada

Discussion

Present Status of the Treatment of Bronchiectasis—William A. Hopkins, M.D., M. Bedford Davis, M.D., and William C. Wansker, M.D., Atlanta, Ga.

Discussion

2:00 p.m.—4:00 p.m.

5B Immunology

An Evaluation of the Agar Double-Diffusion Test in 600 Consecutive Patients—William Lester, M.D., Rosemary Colton, B.A., Robert C. Parlett, M.D., and Guy P. Youmans, M.D., Chicago, Ill.

Discussion

The Effect of Homogenates Prepared from Organs of Normal and Immunized Guinea Pigs on the Respiration of Virulent Tubercle Bacilli—Anne S. Youmans, Ph.D., and Guy P. Youmans, M.D., Chicago, Ill.

Discussion

Immediate Component in Tuberculin Reactions—William D. McKee, M.D., and Cutting B. Favour, M.D., Palo Alto, Calif.

Discussion

A Study of Heaf Test in Comparison with Mantoux Test and Patch Test—Katharine H. K. Hsu, M.D., and Austin E. Hill, M.D., Houston, Texas

Discussion

The Endurance of Positive Mantoux Reactions in Primary Childhood Tuberculosis—William Curtis Adams, M.D., Louisville, Ky.

Discussion

Epidemiologic Factors in Tuberculosis—A Twenty-Four-Year Study—L. D. Zeidberg, M.D., R. S. Gass, M.D., R. H. Hutcheson, M.D., and Ann Dillon, M.S.P.H., Franklin, Tenn.

Discussion

Study of an Outbreak of Tuberculosis aboard the USS Lowe—Jess W. Bromley, M.D., Oakland, Calif.

Discussion

4:30 p.m.—5:30 p.m.

6A Tissue Culture Techniques and Mycobacterial Research

Moderator to be announced

Panelists to be announced

4:30 p.m.—5:30 p.m.

6B Childhood Tuberculosis

Moderator to be announced

Panelists to be announced

4:30 p.m.—5:30 p.m.

Demonstration IV

The Evaluation of Pulmonary Function in Chronic Respiratory Disease—Hurley L. Motley, M.D., University of Southern California Cardiorespiratory Laboratory. Limited to 20 physicians. Reservations must be secured in advance from the American Trudeau Society.

Demonstration V

Pulmonary Evaluation of the Postoperative Patient—Ronald H. Selvester, M.D., C. Joan Coggin, M.D., and Ellsworth E. Wareham, M.D., White Memorial Hospital of the College of Medical Evangelists. Limited to 30 physicians. Reservations must be secured in advance from the American Trudeau Society.

Demonstration VI

The Diagnosis and Management of Acute Respiratory Failure—Herman W. Gierson, M.D., Gino

IOVINE, M.D., HUGH GAFFNEY, M.D., AND JACK HACKNEY, M.D., Los Angeles County General Hospital. Limited to 20 physicians. Reservations must be secured in advance from the American Trudeau Society.

4:30 p.m.—5:30 p.m.

Case Conference

Moderator to be announced

WEDNESDAY, MAY 18

9:00 a.m.—10:45 a.m.

7A Surgery

Vascular Invasion in Lung Cancer—Clinico-pathological Significance—JACK M. MOSELY, M.D., AND DELBERT R. DICKSON, M.D., Santa Barbara, Calif.

Discussion

Isolated Perfusion of the Left Lung—KENNETH JACOBS, M.D., JOHN FLEXNER, M.D., AND H. WILLIAM SCOTT, M.D., Nashville, Tenn.

Discussion

Diagnosis of Acute Pulmonary Embolism by Arteriography—WILLIAM S. STONEY, M.D., AND JESSE E. ADAMS, M.D., Nashville, Tenn.

Discussion

Bronchial Arterial Collaterals—Effect of Experimental Pulmonary Artery Ligation and Subsequent Reanastomosis—HARVEY W. KAUSEL, M.D., L. H. S. VAN MIEROP, M.D., ALLAN S. PECK, M.D., ALLAN STRANAHAN, M.D., AND RALPH D ALLEY, M.D., Albany, N. Y.

Discussion

Topographic Large-Section Study of Satellite Nodules in Pulmonary Coccidioidomycosis—P. J. MELNICK, M.D., AND JOHN D. STEELE, M.D., San Fernando, Calif.

Discussion

Coccidioidomycosis Surgical Data from the V.A.-A.F Cooperative Study—DAVID SALKIN, M.D., San Fernando, Calif.

Discussion

9:00 a.m.—10:45 a.m.

7B Nontuberculous Lung Disease

Rheumatoid Pleurisy with Effusion—DAVID T. CARR, M.D., AND JOHN G. MAYNE, M.D., Rochester, Minn.

Discussion

An Evaluation of Pleural Fluid Glucose Determinations—A. N. RUSSAKOFF, M.D., CHARLES A. LEMAISTRE, M.D., AND HAL J. DEWLETT, M.D., Birmingham, Ala.

Discussion

Microscopic Examination of Bronchial Epithelium in Children—OSCAR AUERBACH, M.D., ARTHUR PURDY STOUD, M.D., E. CUYLER HAMMOND, SC.D., AND LAWRENCE GARFINKEL, M.A., East Orange, N. J.

Discussion

Observations on the Evolution and Course of Sarcoidosis—DAVID REISNER, M.D., Hines, Ill.

Discussion

A Reactant to Human Organ Extracts in Patients with Certain Pulmonary Diseases and Rheumatoid Arthritis—JAMES F. HAMMARSTEN, M.D., ALLEN R. HENNES, M.D., MARGARET Z. MOORE, B.S., AND R. L. CARPENTER, M.D., Oklahoma City, Okla.

Discussion

The Association of Chronic Peptic Ulcer and Chronic Pulmonary Emphysema—GEORGE L. BAUM, M.D., MARTIN H. KALSER, M.D., AND JOHN H. KATHE, M.D., Coral Gables, Fla.

Discussion

The Lungs in Ankylosing Spondylitis—PHILLIP A. ZORAB, M.D., London, England

Discussion

11:00 a.m.—12:00 noon

Lecture

The Concept of Collagen Diseases in Medicine—PAUL KLEMPERER, M.D., New York, N. Y.

Sponsored by the California Trudeau Society

12:30 p.m.—1:45 p.m.

Sandwich Seminar V*

Treatment of Bone and Joint Tuberculosis—ABRAHAM FALK, M.D., Minneapolis, Minn., *Moderator*

Panelists: BARRY FRIEDMAN, M.D., Cleveland, Ohio.

Other panelist to be announced

Sandwich Seminar VI*

Indications for Lung Collapse—NORMAN J. WILSON, M.D., Boston, Mass., *Moderator*

Panelists: HARRY WALKUP, M.D., Oteen, N. C., AND HARRY G. PARSONS, M.D., Weimar, Calif.

Sandwich Seminar VII*

Surgery of Lung Cancer—How Much?

JOHN C. JONES, M.D., Los Angeles, Calif., *Moderator*

Panelists: J. MAXWELL CHAMBERLAIN, M.D., New York, N. Y., ALLAN STRANAHAN, M.D., Albany, N. Y., AND DONALD PAULSON, M.D., Dallas, Texas

Sandwich Seminar VIII*

Treatment of Empyema—PAUL C. SAMSON, M.D., Oakland, Calif., *Moderator*

Panelists: H. WILLIAM HARRIS, M.D., Salt Lake City, Utah, AND JOHN M. SALYER, M.D., Santa Ana, Calif.

2:00 p.m.—3:30 p.m.

8A Symposium on Current Cooperative Studies in Lung Cancer Chemotherapy, Including Experimental Methods and Results

GORDON ZUBROD, M.D., *Moderator*

Participants: JULIUS L. WOLF, M.D., Bronx, N. Y., RAYMOND YESNER, M.D., West Haven, Conn., AND ANTHONY R. CURRERI, M.D., Madison, Wisc.

2:00 p.m.—3:00 p.m.

8B Immunology

An Immunogenic Tubercle Bacillus Extract—ALFRED J. CROWLE, PH.D., Denver, Colo.

Discussion

Experimental Killed Antituberculosis Vaccines Prepared by Solvent Extractions—ANSON HOYT, M.D., MILLIE A. THOMPSON, FREDERICK J. MOORE, M.D., AND C. RICHARD SMITH, M.D., Los Angeles, Calif.

Discussion

The Immunogenicity and Allergenicity of Mycobacterial Cell Walls and Intracellular Particles in CF1 and Strong A Mice—KOOMI KANAI, M.D., GUY P. YOUMANS, PH.D., AND ANNE S. YOUMANS, PH.D., Chicago, Ill.

Discussion

The Influence of Alteration of the Diet on the Susceptibility of the Albino Rat to Mycobacterium Tuberculosis—RICHARD L. COSTELLO, M.A., LOYD W. HEDGECOCK, PH.D., AND TOM R. HAMILTON, M.D., Kansas City, Mo.

Discussion

Serologic and Skin Test Activity of Polysaccharides Derived from Systemic Mycotic Pathogens—RALPH A. KNIGHT, PH.D., GILBERT A. HILL, PH.D., AND STANLEY MARCUS, PH.D., Salt Lake City, Utah

Discussion

Drug Treatment of Pulmonary Tuberculosis

An Interim Report by the Committee on Therapy

The Committee on Therapy has reviewed the statements on the treatment of tuberculosis published in 1955 and in 1958 (1, 2). Developments that have occurred since 1958 were thought to be of sufficient interest for an interim progress report at this time.

The principal developments relate to: (*a*) single-drug therapy in selected patients, (*b*) combinations of three drugs versus two, (*c*) isoniazid in high dosage, (*d*) pyrazinamide, (*e*) streptomycin-pantothenate, (*f*) streptovaricin, (*g*) cycloserine, (*h*) thiocarbaindin, (*i*) kanamycin,

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and (*j*) adjuvant adrenocorticosteroid and corticotropin therapy in tuberculosis. Since there have been no recent developments relative to viomycin, its role remains secondary, and its use as a companion drug is recommended when combinations of other drugs cannot be used for one reason or another.

Single-drug therapy with isoniazid: Isoniazid in adequate dosage, combined with one or more of the other antituberculous drugs, is the currently accepted drug regimen of choice. However, studies of the short-term (four to eight months) effectiveness of isoniazid alone as compared with isoniazid plus PAS indicate that isoniazid alone is as effective as isoniazid plus PAS in the treatment of minimal and moderately advanced noncavitary disease (3). It remains to be determined whether isoniazid alone will suffice for long-term therapy of this type of tuberculosis. The use of isoniazid alone offers the advantages of better patient acceptance of medication and lower cost of treatment. It also remains to be determined by carefully controlled studies whether, under other conditions, such as in a patient with a single small cavity or possibly even slightly more advanced disease, isoniazid alone is therapeutically as effective as isoniazid plus PAS. No such evidence is currently available.

Combinations of three drugs versus two: The therapeutic superiority of a regimen of three drugs (streptomycin, PAS, and isoniazid) over two-drug regimens containing isoniazid has not been demonstrated by clinical studies (4). Moreover, the addition of a third drug to a two-drug regimen arithmetically increases the toxicity. However, it has been reported, but not confirmed, that in resected lesions fewer tubercle bacilli resistant to drugs were found when a triple-drug combination has been used than when a two-drug regimen has been followed.

Isoniazid in high dosage: Can the therapeutic efficacy of isoniazid be improved by increasing the conventional adult dosage of 3 to 5 mg. per kg. per day to a higher dosage of 12 to 24 mg. per kg. per day? The technique of performing urine and blood serum bioassays of active isoniazid is being more widely adopted in many laboratories. Clinically, more has been learned concerning the characteristics of slow and rapid inactivators of isoniazid. There is need to determine whether in either group, but particularly in the rapid inactivator group, higher dosages of isoniazid improve its efficacy. Genetic factors play an important role in the metabolic disposition of isoniazid. Knowledge of the inactivation pattern of isoniazid at the beginning of therapy may enable one to predict which patients may fail to respond to treatment. Therapy should be modified accordingly. It is recommended that, when the dosage of isoniazid is increased, a minimum dosage of 50 mg. of pyridoxine per day be added to protect against possible isoniazid toxicity.

Pyrazinamide: Pyrazinamide used alone has only a limited effect. Its potential for serious hepatotoxicity, necessitating frequent liver-function tests, restricts its use to hospitalized patients. It is not recommended for long-term therapy. The oral adult dose of pyrazinamide is 1 gm. three times daily. Doses lower than this, although they reduce its toxic effect, appear to compromise the drug's therapeutic effectiveness (5).

Streptomycin-pantothenate: The use of streptomycin-pantothenate as a substitute for streptomycin-sulfate does not reduce the damage to the eighth cranial nerve. This observation confirms the unfavorable reports on streptomycin-pantothenate published in the European literature. On the other hand, most of the favorable reports in the European literature were in regard to the effective use of dihydrostreptomycin-pantothenate as compared with dihydrostreptomycin-sulfate in reducing the toxicity of the eighth nerve. The few reported attempts in the United States to evaluate dihydrostreptomycin-pantothenate have not supported this finding (6). Dihydrostreptomycin-pantothenate is believed to offer no advantage over other streptomycin or dihydrostreptomycin drugs.

Streptovaricin: Streptovaricin is a product of *Streptomyces spectabilis* isolated from soil near Dallas, Texas. In animal experiments, streptovaricin enhanced the action of isoniazid to a greater degree than any other companion drug tested, except pyrazinamide. In human trials, streptovaricin in a single oral dosage of 3 gm. per day is poorly absorbed, and in combination with isoniazid (300 mg. per day) has no therapeutic advantage over isoniazid alone. Streptovaricin in a dosage of 10 gm. per day can be tolerated. However, it is not known whether its therapeutic efficacy can be enhanced by this higher dosage.

Cycloserine: Cycloserine has been demonstrated to be an active drug in the treatment of previously untreated cases of pulmonary tuberculosis. Administered orally to adults in dosages of 0.5 gm. twice daily, it causes convulsions in approximately 8 per cent of the patients treated. This toxicity has been reduced significantly by lowering the dosage to 0.25 gm. twice daily. In combination with isoniazid, cycloserine in this lower dosage is approximately as effective as isoniazid plus PAS except in patients with far advanced cavitary disease. In view of this, cycloserine might be used as a substitute for PAS. Under certain circumstances, the toxic effects of high doses of cycloserine on the central nervous system have

been reported to be controlled, if not prevented, by concomitant administration of sedatives, anticonvulsants, tranquilizing agents, and pyridoxine, singly or in combinations (7). In very difficult clinical situations, when other treatment resources have been exhausted, trial dosages of cycloserine higher than 0.25 gm. twice daily are clinically justified. The combination of cycloserine with pyrazinamide has also been proposed as worthy of further study.

Thiocarbanidin: Thiocarbanidin, a thiocarbanilide derivative, is active against experimental tuberculosis in mice and guinea pigs. Cross resistance between thiocarbanidin and amithiozone has been demonstrated *in vivo*. Thiocarbanidin is poorly absorbed from the human gastrointestinal tract. In man, a single oral dose of 1 gm. of thiocarbanidin per day is less effective than PAS in delaying resistance to isoniazid and probably to streptomycin. Since the lack of therapeutic efficacy may be related to poor absorbtion and low blood concentrations, attempts are being made to produce a more assimilable form of thiocarbanidin to achieve adequate serum concentrations.

Kanamycin: Kanamycin, a broad-spectrum antimicrobial, isolated from the culture broths of *Streptomyces kanamyceticus* by Ueda and associates in Japan in 1957, is effective against experimental and human tuberculosis. Kanamycin is closely related to neomycin. Resistance to kanamycin develops quite rapidly. Cross resistance to neomycin and to viomycin has been demonstrated. At a dosage of 1 gm. per day (0.5 gm. twice daily intramuscularly) auditory toxicity is a common and serious complication of prolonged therapy. Renal toxicity, manifested by proteinuria or cylindruia, frequently occurs. According to investigators in Japan, reduction of the total weekly dosage, not to exceed 4 gm., may delay or prevent toxicity to the eighth nerve while still retaining therapeutic efficacy, but this has not yet been confirmed in this country. When kanaymcin was used in moderate dosages for a short period of time for the treatment of nontuberculous infections, no serious adverse effects have been observed.

Adrenocorticosteroids and corticotropin: Beneficial effects from the addition of adrenocorticosteroids and corticotropin have been reported in the treatment of tuberculous meningitis, miliary tuberculosis, pleurisy with effusion, and hypersensitivity to antituberculous agents. The mechanism by which the hormones may reverse an unfavorable course and perhaps accelerate the improvement beyond what may be expected from antimicrobial therapy alone remains obscure. The phenomenon is not totally explained by antiinflamatory effect, by adrenocortical replacement in adrenal exhaustion, or by alterations that occur at the cellular level to make the tuberculous infection more susceptible to drug treatment. A large body of recorded clinical experience indicates that adjuvant hormone therapy is less dangerous than had been feared. Further evidence obtained through controlled studies is needed to clarify the role of adrenocorticosteroids and corticotropin in the treatment of selected tuberculous patients whose organisms are susceptible to drugs.

THOMAS B. BARNETT
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GARDNER MIDDLEBROOK
DONALD L. PAULSON
JAMES W. RALEIGH
WILLIAM W. STEAD
JAMES A. WIER, *Chairman*

REFERENCES

(1) AMERICAN TRUDEAU SOCIETY: Am. Rev. Tuberc., 1955, *72*, 408.

(2) AMERICAN TRUDEAU SOCIETY: Am. Rev. Tuberc., 1958, *78*, 656.

(3) PHILLIPS, S.: Results of original course of isoniazid chemotherapy for minimal or noncavitary, moderately advanced tuberculosis, Tr. Seventeenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1958, p. 112.

(4) LIVINGS, D. G.: Results of original course of chemotherapy for tuberculosis: Data from cooperative study, general regimens, Tr. Eighteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1959, p. 18.

(5) MATTHEWS, J. H.: Pyrazinamide–INH in the treatment of pulmonary tuberculosis: Data from cooperative studies 11 and 18, Tr. Eighteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1959, p. 106.

(6) DONOHOE, R. F., *et al.*: Daily dihydrostreptomycin-pantothenate combined with massive doses of Salizid® in the treatment of pulmonary tuberculosis, Tr. Seventeenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1958, p. 349.

(7) PYLE, M. M., *et al.*, Cycloserine in high dosage in "salvage cases" of pulmonary tuberculosis with control of toxicity by concomitant medication: Efficacy as medical and presurgical treatment, Tr. Eighteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1959, p. 128.

Chemotherapy of Extrapulmonary Tuberculosis in Adults

A Statement of the Committee on Therapy

The management of pulmonary tuberculosis varies considerably to meet problems of individual cases, but the following general principles of chemotherapy, which are widely followed, are also believed to apply to many forms of extrapulmonary tuberculosis:

1. Antibacterial chemotherapy is recommended for all patients with active tuberculous infection.
2. Chemotherapy should be intensive and uninterrupted. Most physicians, but not all, advise combined chemotherapy, using isoniazid with either PAS or streptomycin. When it is well tolerated, PAS is usually employed because it is easily administered. Some physicians prefer to use three drugs, isoniazid, PAS, and streptomycin, for more severe manifestations of tuberculosis. The usual dosage of isoniazid is 100 mg. three times daily (3 to 5 mg. per kg. of body weight), but there is growing indication that larger doses are sometimes more beneficial. Larger doses require the administration of pyridoxine to minimize the risk of neurologic complications (peripheral neuritis). Para-aminosalicylic acid is given in maximal tolerated doses, ordinarily 4 gm. three times daily (150 to 200 mg. per kg. of body weight). Daily administration of streptomycin, 1.0 gm. (15 to 20 mg. per kg. of body weight), is ordinarily reserved for severe manifestations of tuberculosis, and injections every second or third day appear adequate in many cases.
3. Chemotherapy is continued for a prolonged period—usually two years and at least one year—after the tuberculosis has become inactive, as determined by roentgenographic, bacteriologic, and clinical diagnostic methods.
4. Surgical treatment for pulmonary tuberculosis, particularly pulmonary resection, is ordinarily delayed for at least several months. This long preliminary course of medical treatment sometimes obviates the need for surgery, usually diminishes the risk of tuberculous complications of surgery, and sometimes permits more conservative operations than would have been necessary earlier in the course of the illness. At other times lesions that appear to be inoperable improve substantially as a result of medical treatment and become operable. In some forms of extrapulmonary tuberculosis, it is probable that surgery, particularly the radical procedures, should be similarly delayed for several months after medical treatment is begun.

Skeletal tuberculosis: When affected by tuberculosis, a weight-bearing joint is more likely to require surgical fixation than a non-weight-bearing joint. Peripheral joints heal more rapidly and completely than proximal joints. Joints of the spine are more difficult to treat than joints of the extremities.

Tuberculosis which involves only the synovial membrane is often completely reversible, and a functional cure is usually possible with chemotherapy. Early diagnosis of such a condition may require biopsy.

The fusion of tuberculous joints is best delayed until medical treatment has diminished soft tissue reaction, closed any draining sinuses present, and stabilized the pathologic process, and may be avoided altogether in many instances.

Tuberculous abscesses should be evacuated when possible to facilitate healing.

Tuberculous lymphadenitis: Tuberculous lymph nodes often resolve rather slowly under treatment with specific drugs, but eventual healing is usually possible without surgery. Abscesses associated with tuberculous lymph nodes should be evacuated (aspirated) and occasionally resected. Acid-fast bacilli that are not typical *M. tuberculosis* are occasionally found to cause lymphadenitis. These organisms are usually not responsive to antituberculous drugs.

Genitourinary tract tuberculosis: Renal tuberculosis demands prolonged medical treatment in all cases—for even longer periods than in the case of pulmonary diseases—and results are frequently satisfactory, even without resection of seriously damaged kidneys. Opinion among surgeons is divided as to what constitutes an indication for nephrectomy. Tuberculous cystitis (always a consequence of renal tuberculosis) responds promptly when the infection is recent and superficial. Despite early clearing of symptoms of cystitis, prolonged treatment is essential for permanent results.

Tuberculous epididymitis, seminal vesiculitis, and prostatitis respond slowly but definitely to adequate and prolonged chemotherapy.

Tuberculosis of the female genital tract frequently requires surgery in addition to chemo-

therapy, especially removal of the abscesses associated with tuberculous salpingitis. Tuberculosis of the endometrium is usually secondary to tuberculosis elsewhere in the genital tract, but appears to respond to medical treatment.

Miliary tuberculosis and tuberculous meningitis: These forms of tuberculosis may frequently be treated successfully, especially when early intensive and prolonged therapy is administered. Isoniazid is considered the most important drug. Streptomycin is usually employed daily for a few weeks or months. There is growing evidence that the adrenal corticosteroid hormones are beneficial. Intrathecal therapy rarely is used in the treatment of tuberculous meningitis.

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Treatment of the Adult Tuberculin Converter

A Statement of the Committee on Therapy

During the first few years of the past decade there was at first a cautious and then a very rapid broadening of the indications for specific antimicrobial therapy for tuberculosis. This led to the current consensus that such therapy should be included in the treatment of all forms of active tuberculosis. More recently the indications have been further broadened to include certain persons who have no evidence of tuberculosis other than positive tuberculin reactions.[1] There would appear to be more than adequate justification for the now widespread use of antimicrobial therapy in infants with positive tuberculin reactions in view of the incidence and serious nature of the complications of tuberculosis at this age. Reports of studies designed to establish the place for antimicrobial therapy in the adult tuberculin converter are not yet available, and those dealing with children are preliminary in that they pertain only to early observations (1). Nevertheless, it seems appropriate to consider the matter of the treatment of the adult tuberculin converter in the light of the information available.

Tuberculin conversion as used in this statement indicates the appearance of hypersensitivity to tuberculin in an individual known to have been a nonreactor in the recent past. Every individual in whom tuberculin hypersensitivity appears should be studied—by means of a complete medical history and physical examination, a roentgenogram of the chest, and cultures of three specimens of sputum or gastric contents—for evidence of a clinical infection. If such studies reveal other evidence of tuberculosis, appropriate treatment should be instituted. This statement will deal with those in whom the only evidence of tuberculous infection is recent tuberculin conversion.

The appearance of a positive tuberculin reaction at any age indicates a tuberculous infection that may or may not result in clinical tuberculosis. Before concluding, however, that a recent tuberculin conversion is an indication for antimicrobial therapy, the physician must determine (*1*) the risk to the individual without antimicrobial therapy, (*2*) the effect of therapy on this risk, and (*3*) the undesirable effects of the therapy.

The reported morbidity, both early and late, in the adult known to have converted the tuberculin reaction from negative to positive varies widely. Myers and others (2) reported the development of other manifestations of tuberculosis in about 5 per cent of a group of student nurses who were known tuberculin converters and who were followed for periods of four to twenty-eight years. Many of these had "primary pulmonary infiltrates" and pleural effusions without subsequent tuberculous developments. At the other extreme, Hyge (3) found tuberculosis in some form in 60 per cent of a group of adolescent students followed for twelve years after converting the tuberculin reaction as a result of a classroom exposure. There have been many other reports (4–12) dealing with such observations, and most of these would suggest that the risk of developing tuberculosis following conversion of the tuberculin reaction is between 5 and 15 per cent with even higher figures reported in some groups. The reasons for these wide variations are not entirely clear. Differences in selection of cases and techniques of follow-up observation may account for some of these variations, and it seems likely that the nature and frequency of exposure and the susceptibility of the individuals to tuberculosis would account for some of these differences.

Very little information is available about the effect of antimicrobial therapy on morbidity following conversion of the tuberculin test. There is evidence that the administration of antimicrobials to tuberculin-positive children (1) and to patients with tuberculous pleural effusion (13, 14) very significantly alters the ultimate prognosis and markedly lowers the incidence of complications. This difference is most marked in those cases in which isoniazid is included in the therapeutic program. From these facts and from the vast experience with chemotherapy of established tuberculosis, it seems reasonable to expect that the institution of antituberculous drugs following conversion of the tuberculin test would significantly reduce the incidence of tuberculous disease. This is not to say that the risk would be altogether eliminated.

The incidence and type of drug toxicity would depend on the therapeutic agent used, but this should rarely amount to a serious problem. Regardless of the choice of drugs, the mere fact that treatment is advised might produce some anxiety on the part of the patient and his family; if the situation is properly handled by the physician and the reasons for recommending therapy are

[1] As defined by the Committee on Tuberculosis in Children of the American Trudeau Society.

carefully explained, anxiety would not appear to be of major importance.

There is insufficient evidence available at the present time to allow any definite or final conclusions about the use of antimicrobial drugs in adult tuberculin converters. Nevertheless, in the light of the above considerations, one can justify this form of therapy in known recent converters. The indications for such a course of action are even more definite in the following converters: (*1*) those who for some reason are thought to be unusually susceptible to tuberculosis, such as adolescents and persons with diabetes or silicosis; (*2*) those known to have had unusually heavy or prolonged exposures to tuberculosis; and (*3*) others in whom unusual circumstances would indicate every precaution in preventing clinical tuberculosis.

The absence of careful and controlled comparative clinical studies also precludes final statements regarding the proper choice of drugs in the cases in which treatment seems indicated. In the treatment of established tuberculosis it remains the opinion of most that multiple-drug therapy offers real advantage over therapy with a single drug (isoniazid), especially in advanced pulmonary disease and in most extrapulmonary disease. For this reason multiple-drug therapy in the tuberculin converter might be expected to afford maximal assurance of preventing clinical tuberculosis. If two drugs are used in such cases, isoniazid and PAS are suggested as the combination of choice. Since there is some evidence that, in the early months at least, isoniazid alone compares favorably with isoniazid and PAS in the treatment of minimal and moderately advanced pulmonary tuberculosis without cavities (15), the use of this single drug in the recent tuberculin converter might be a satisfactory alternative therapeutic regimen. The use of isoniazid alone is convenient, inexpensive, and relatively free from toxic side effects. Including PAS in the treatment would make for less convenience, greater expense, and greater incidence of side effects that could be expected to increase the interruptions in the program and the chances of discontinuing both drugs prior to completion of the prescribed course. The physician in charge must balance the advantages and the disadvantages of giving PAS plus isoniazid rather than isoniazid alone, but whatever the choice of drugs, it is recommended that treatment be continued without interruption for a period of at least one year on an ambulatory basis.

The decision to use antimicrobial therapy in the recent tuberculin converter does not lessen the importance of close observation of the individual being treated. In addition to the necessary examinations to detect manifestations of tuberculosis, such persons must be observed for evidence of drug toxicity. The development of a positive tuberculin reaction, especially in someone not known to be in contact with tuberculosis, should be followed up with careful examination of family and close contacts to discover, if possible, the source of the infection.

The use of antimicrobial agents in the case of a recent tuberculin converter is designed as a measure to prevent clinical tuberculosis and does not mean that the patient has a disabling disease. When a hospital employee develops a positive tuberculin reaction, the matter of reporting this to industrial accident commissions should be based on general policies applying to tuberculin converters and should not be affected by the fact that therapy has been begun.

It should be re-emphasized that the place for antimicrobial therapy in the management of the recent tuberculin converter is not yet finally established. Careful, controlled clinical studies should be carried out in an effort to establish more firmly the advantages of such therapy.

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Treatment of Tuberculosis in Children

A Statement by the Committee on Tuberculosis and Respiratory Diseases in Children

Although extensive comparative studies on the use of chemotherapeutic agents in childhood tuberculosis have not been carried out on a randomized basis, as has been done with adults, the committee thinks that it is possible, on the basis of individual experiences and information derived from adult studies, to make certain recommendations.

Use of Drugs

Isoniazid: Isoniazid should be a part of every regimen for treatment of *active* tuberculosis. Although the usual dose of isoniazid for adults is 4 to 5 mg. per kg., doses as high as 30 mg. per kg. per twenty-four hours have been used for children without complications. It is not certain that such large doses are required; a more usual level is about 15 mg. per kg. daily taken in two or three doses.

A difference of opinion with respect to the proper dosage is due to the fact that in children, as in adults, a certain (at present unknown) proportion of patients rapidly inactivates the drug and renders it impotent by conjugation in the liver. To those who have used the larger doses of isoniazid, pyridoxine has not seemed to be a necessary additional drug for the prevention of neuritis in children, although it may be necessary in adolescents.

PAS: Para-aminosalicylic acid is the most commonly used second drug when a combination regimen is employed. The dosage is usually 200 mg. per kg. per twenty-four hours. When salts, sodium, potassium, or calcium para-aminosalicylate are used, the dose should be correspondingly larger, such as 300 mg. per kg. daily. In general, children have a much better tolerance for all forms of PAS than do adults.

PAS apparently competes with isoniazid for conjugation by the liver and should be a part of the regimen when the dose of isoniazid is in the 4 to 5 mg. per kg. range. The advantage of a double-drug regimen lies in the fact that the emergence of resistant strains of *M. tuberculosis*, which takes place during rapid multiplication of organisms, is delayed or prevented.

Streptomycin: Streptomycin is not used except under special conditions. The drug is undesirable because it must be administered intramuscularly and because it is toxic, especially to the eighth nerve. The usual dose is 20 mg. per kg. per day.

Dihydrostreptomycin should not be used in pediatric practice, since it has a selective and often irreversible toxicity for the auditory division of the eighth nerve and since adequate audiometric studies may be impossible in the very young.

The newer antituberculous drugs—such as viomycin, cycloserine, and pyrazinamide—usually have no place in the treatment of tuberculous children.

Triple-drug therapy: It has not been conclusively demonstrated that administration of isoniazid, PAS, and streptomycin simultaneously (so-called "triple-drug therapy") has a definite advantage over any two-drug combination that includes isoniazid, and it should be kept in mind that toxicity is increased proportionately. Therefore, "triple-drug therapy" should be reserved for the more serious forms of disease, as discussed below.

Corticosteroids: Corticosteroids should be used for the infant or young child who is dangerously ill in order to support him until antimicrobial drugs have an opportunity to produce some effect. Their routine use cannot be recommended at this time. The indications for the use of corticosteroids in pleurisy, meningitis, and endothoracic primary complex will be discussed below. The usual dose is 1 mg. per kg. of prednisone per day for two to three months with gradual withdrawal.

Duration of Therapy

The optimal duration of therapy is not known with precision, but the usual duration of drug therapy is at least one year and preferably eighteen or even twenty-four months, depending on the extent and severity of the disease, the longer duration being reserved for more serious forms of disease. When streptomycin is employed in a triple-drug regimen, it should be discontinued within a month after satisfactory clinical response.

Types of Disease

Endothoracic Primary Tuberculous Complex

Enlarged tracheobronchial lymph nodes and disease due to bronchial obstruction and/or bronchogenic spread are the most common forms of endothoracic tuberculosis in children. More extensive involvement may occasionally be seen. Drug therapy consisting of isoniazid and PAS should be continued for a minimum of one year.

Roentgenographic findings may indicate that more prolonged therapy is necessary. Fresh pulmonary infiltrations respond quickly to drug therapy. Large mediastinal nodes and segmental involvement usually show slow changes. When endomural granuloma is the cause of obstruction, bronchoscopic removal of the granuloma may hasten the re-aeration of the segment involved. In young children with intense dyspnea caused by tuberculous bronchitis and tuberculous mediastinal nodes, marked relief may be obtained very quickly through the addition of corticosteroids.

Tuberculous Meningitis

It is the opinion of the committee that all patients with tuberculous meningitis should receive triple-drug therapy, streptomycin being given daily (at least for a few weeks) and isoniazid in comparatively high dosage. Certainly the severely ill child, i. e., the child in coma or with suspected block, should be treated with corticosteroids; and some clinicians hold the view that all children with tuberculous meningitis should receive corticoids. There has not yet been sufficient controlled observation in children to permit the flat statement that all should receive corticosteroid therapy. The proper duration of therapy in cases of meningitis is unknown, but it would appear that treatment with isoniazid and PAS should be continued for a minimum of one year and in many cases longer, although streptomycin should be discontinued within a month after satisfactory clinical response.

Miliary Tuberculosis

Once again, triple therapy with fairly high doses of isoniazid and streptomycin is thought to be indicated during the first part of illness. Streptomycin may be discontinued after the temperature becomes normal. The patient should then continue on isoniazid and PAS for at least a year, although the optimal duration of therapy is unknown.

The corticosteroids may be used to assist in relieving extreme dyspnea associated with miliary disease.

Extrathoracic Tuberculosis

It is the opinion of this committee that all superficial and accessible abscesses caused by tubercle bacilli should be drained as freely and as readily as the abscesses caused by other organisms. Local excision of tuberculous tissue is perfectly safe provided the patient is receiving effective antituberculous chemotherapy.

Renal tuberculosis: The discovery of tubercle bacilli in culture of the urine, even though pyelograms reveal no evidence of deformity, calls for treatment in all cases. The reported evidence suggests that all but the very minimal cases of renal tuberculosis require a triple-drug regimen and that the duration of treatment should be two years.

Superficial lymphadenopathy: The general problems of drainage and excision are re-emphasized in cases of superficial lymphadenopathy. The preferred treatment is drug therapy, which may be followed by local excision. Some physicians prefer to aspirate pus and to administer corticosteroids as well as antimicrobial agents; others rely entirely on the antimicrobial drugs, principally isoniazid and PAS. Experience indicates that the duration of therapy should be at least twelve months.

Tonsillectomies and adenoidectomies are not performed routinely but should be considered on the basis of the individual problem.

Tuberculosis of bone and joint: The same principles of drug therapy and local excision and drainage enunciated above apply to these conditions. It is thought that in the non-weight-bearing structures immobilization is not necessary and may be detrimental. In the case of weight-bearing structures it is thought that the main problem is prevention of weight-bearing and only now and then will prolonged immobilization be necessary. In the extremities, fusion should be avoided whenever possible.

Treatment always calls for isoniazid, usually associated with PAS. Some believe that triple-drug therapy should be used in bone and joint tuberculosis. Treatment should continue for eighteen to twenty-four months in all cases.

Serous effusions: All effusions should have diagnostic aspirations, and some clinicians advocate fairly complete aspiration of fluid. All patients with these manifestations of tuberculosis should receive a regimen containing isoniazid for a minimum of twelve months. Some physicians feel that the addition of steroids has some advantage in preventing pleural thickening and adhesions and particularly pericardial adhesions.

Pulmonary Tuberculosis of the Adult Type in Children

Pulmonary tuberculosis in a child presents essentially the same problem as in adults. There is perhaps a higher incidence of this form of tuberculosis in pubertal and prepubertal girls. The same general principles regarding cavity closure and sputum "conversion" apply here as in adult tuberculosis, and the problems of the management of the open cavity are essentially the same. Thoracoplasty should by all means be

avoided, although excisional surgery is often indicated.

So-called Prophylactic Treatment of the Tuberculin Converter with no Manifest Disease

If tuberculin conversion is known to have occurred within one year prior to the time of examination, it is the opinion of the majority of the committee that all children up to fourteen years should be placed on treatment, using either one or two drugs, one of which should certainly be isoniazid. The treatment should be continued for at least one year and possibly longer. Isolation and bed rest are not necessary.

When the date of conversion of the tuberculin reaction is unknown and the patient has no signs or symptoms, it is thought that for maximal safety all patients less than 36 months should be treated for at least one year. Intercurrent infections, such as measles and pertussis, increase the hazard and therefore the need for antimicrobial therapy. It is also thought that the larger the initial tuberculin reaction the greater the risk of that child's developing active tuberculous disease.

Both recent converters and those whose conversion date is unknown should remain under careful observation for many years and should be closely followed in the event of intercurrent infections and as the patients approach the age of puberty.

Any tuberculin-positive child who is to receive steroid treatment for another disease should receive concurrent antituberculous treatment.

A child with a positive tuberculin reaction should receive antituberculous therapy for at least four weeks in the event of an attack of measles.

Tuberculin Testing

The committee recommends that all children should be tested intradermally with a material equivalent to 5 TU of PPD-S. The first test under ordinary circumstances should be carried out at six to eight months of age; if that test is negative, the test should be applied at least once a year thereafter, unless the child is known—or suspected—to have been exposed to tuberculosis.

In the event of known or suspected exposure to an open case of tuberculosis, a tuberculin test should be administered as soon as possible. If it is negative, it should be repeated as often as every four weeks until it becomes positive or until the longest possible period—three or four months—for the development of tuberculin allergy has passed. In the event that the contact with tuberculosis continues, the child should be tested as often as every month until contact ceases or until the tuberculin test converts. It is not yet certain whether the administration of isoniazid to an infant or young child who remains in constant contact with a case of tuberculosis, but whose tuberculin skin test is negative, will prevent infection or whether the drug merely suppresses infection and postpones the development of tuberculin sensitivity until drug administration is stopped.

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NOTICES

The following dates and locations of the next five annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California—May 15–20
1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

FORTY-FIFTH ANNUAL SESSION OF THE TRUDEAU SCHOOL OF TUBERCULOSIS AND OTHER PULMONARY DISEASES—1960

The Trudeau School of Tuberculosis and Other Pulmonary Diseases, which will hold its Forty-fifth Session from June 6 to 24, 1960, continues to provide a unique opportunity for training in the field of chest diseases. This annual postgraduate course, conducted under the auspices of the Trudeau Foundation and supported by the Hyde Foundation, is able to provide outstanding instruction at a minimal tuition of $100.00 for a three-week session. Attendance at the Trudeau School carries with it some distinction as well as a thorough review for specialization in pulmonary diseases or for work in public health involving tuberculosis.

In addition to the local medical faculty, consisting of some forty doctors from Saranac Lake, Ray Brook State Tuberculosis Hospital, and the Sunmount Veterans Administration Hospital, about thirty of the leading teachers and investigators in the eastern United States and Canada are brought to Saranac Lake each year to lecture or to conduct seminars in their special fields.

Approximately half of the time is devoted to tuberculosis and the other half is divided between such subjects as silicosis, pulmonary fibrosis, emphysema, fungal infection, sarcoidosis, pneumonias, and intrathoracic tumors.

The Trudeau School, in its environment, gives its students a chance to combine a great educational experience with an enjoyable holiday in the most beautiful part of the Adirondacks, among pleasant companions. The enrollment is necessarily limited and, therefore, application should be made early. A few scholarships are available for those who qualify.

All inquiries should be addressed to the Secretary, Trudeau School of Tuberculosis and Other Pulmonary Diseases, Box 500, Saranac Lake, New York.

LUNCHEON FOR DR. JAMES J. WARING

The Denver Tuberculosis Society will honor Dr. James J. Waring at the Society's annual luncheon meeting on Thursday, April 21, 1960, at 12:00 noon, in the Silver Room of the new Hilton Hotel in Denver. "Dr. James J. Waring—This is Your Life" will be the title of this meeting honoring one of Colorado's most eminent physicians for his contribution over a lifetime in the fight against tuberculosis. At this meeting, the Society will present Dr. Waring with a volume of messages and greetings from his many friends. To assist us in preparing this volume of letters and telegrams, we are asking that all messages to Dr. Waring be sent to the Denver Tuberculosis Society, 728 15 Street, Denver 2, Colorado.

SOUTHWEST ALLERGY FORUM

The Southwest Allergy Forum will be held in Oklahoma City at the Skirvin Hotel from April 2 through April 5, 1960. All allergists in the area are invited to attend. For further information write to George L. Winn, M.D., Secretary, 711 Northwest 10th, Oklahoma City, Oklahoma.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE

VOLUME 81 March 1960 ABST. No. 3

The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 40 abstracters systematically review 207 journals which represent 31 countries and which are published in 18 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title).

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Ruptured Aneurysm of the Inferior Thyroid Artery Associated with Paralysis of the Vocal Cord. Report of Case. A. V. Doumanian*, E. H. Soule, and F. H. Ellis, Jr. *Proc. Staff Meet. Mayo Clin.*, June 10, 1959, 34: 303-309. (*Mayo Clinic, Rochester, Minn.)

A case is reported of paralysis of the right vocal cord due to an aneurysm of the right inferior thyroid artery. Review of the literature of the past 20 years failed to disclose a similar case. The general subject of vocal cord paralysis is reviewed.

E. A. Riley

Considerations on the Psychosomatic Aspects of Bronchial Asthma (in German). B. Staehelin*. *Schweiz. med. Wchnschr.*, May 23, 1959, 89: 560-563. (* Kantonsspital, Zürich, Switzerland)

The psychosomatic approach in 2 asthmatic patients showed that allergic reaction could not be explained by pathophysiology alone without the integration of its significant biographic meaning. The asthmatic patient lives in a world full of menace. His predominant—although generally unconscious—feeling is anxiety. This state of mind is also going to have considerable influence on his behavior—his general outlook as well as the physical state of his body. The asthmatic patient is thus entirely "outlined" on a state of fearful vigilance and suspicion. His proneness to allergic and asthmatic reactions is a symptom of this generalized sensibilization; these reactions often constitute a necessary system of alarm and defense within the somatic sphere. Therapeutic consequences are briefly reviewed (after author's summary).

J. Haapanen

Chronic Bronchitis and Its Treatment (in German). W. Seitz*, N. Zöllner, and J. Meier. *Deutsche med. Wchnschr.*, June 19, 1959, 84: 1125-1129. (*U. of München, Germany)

Exfoliative Cytology in Metastatic Cancer of the Lung. B. F. Rosenberg, H. J. Spjut, and M. M. Gedney*. *New England J. Med.*, July 30, 1959, 261: 226-231. (*Barnes Hosp., St. Louis, Mo.)

No reports devoted solely to an appraisal of cytologic studies of metastatic lung lesions were found in the literature. Fifty cases of metastatic lung cancer, including the clinical histories, the surgical or autopsy material, and the cytologic smears, are reviewed. Nineteen patients (38 per cent) were found on review to have positive cytologic results, and an additional 5 had suspicious smears. Four cases are summarized in which the cytologic results made possible a definitive diagnosis of a metastatic lesion in the lung. A fifth case, not one of the series included herein, is reported to demonstrate the usefulness of accurate typing. In this case, the typing of the malignant cells in the sputum as epidermoid carcinoma ruled out a metastasis from a suspected renal-cell carcinoma and permitted prompt definite surgical treatment for primary carcinoma of the lung.

The percentage of positive results from exfoliative cytology in this group of patients, although low, is encouraging. Furthermore, the typing of these lesions may have an important bearing on the clinical picture: correspondence of cytologic type with that of the suspected primary lesion helps to confirm the diagnosis of metastatic disease. Discrepancy in type from a suspected primary source (as in the fifth case report) may indicate that the pulmonary lesion is actually primary.

These findings indicate a definite place for the cytologic examination of sputum or bronchial washings in all patients with cancer who have pulmonary symptoms or an asymptomatic lesion in the chest on roentgenographic study.

M. J. Small

The Importance of Early Diagnosis of Bronchogenic Carcinoma: Clinical Analysis of 236 Cases of Bronchogenic Cancer in the Municipality of Shanghai. M. H. Shih*, S. Y. Ku, C. P. Chang, K. S. Ku, Y. T. Tiao, and E. M. Chu. *Chinese M. J.*, July, 1959, 79: 19-25. (*Shanghai Med. College, China)

Two hundred and thirty-six cases of proved bronchogenic carcinoma were observed over an 8-year period in 16 hospitals; 83 per cent of the patients were past 40 years of age at the time of admission, although the youngest patient was only 19 years. The ratio of males to females was 3.5:1.

Early cases had no typical symptoms. With more advanced disease, the most common symptoms were cough, blood-streaked sputum, and chest pain. Thirty-two cases were first misdiagnosed as pulmonary tuberculosis. Forty-one per cent of these patients were inoperable when first seen; 3 per cent refused operations, and 26 per cent of the original group were simply explored; 30 per cent of the original group had resection, but only half of these could undergo radical resection.

Exploratory thoracotomy should be given more consideration in cases in which the nature of the pathologic changes is undetermined, but where the possibility of lung cancer cannot be excluded.

L. Hyde

Respiratory Manifestations of Collagen Diseases (in French). A.-P. JARNIOU*, H. BAYLON, A. MOREAU, and M. ENJALBERT. *Semaine hôp.*, June 18, 1959, 35: 464–471. (*Clamart, Seine, France)

This is a report of 2 cases belonging in the group of collagen diseases and showing, in addition to multiple visceral manifestations, specific pulmonary lesions on the chest roentgenogram. One case was diagnosed as periarteritis nodosa, and the other as scleroderma. A general survey is given on respiratory disorders observed in cases of collagen diseases. Pulmonary changes caused by bacilli and observed in the course of a collagen disease have to be excluded. In order to interpret the pulmonary lesions as manifestations of the collagen disease, the diagnosis has to be supported by clinical and histologic evidence. This proves to be rather difficult in many cases.

E. LYON

A Case of Tracheal Cyst (in Italian). L. DEL TORRE*. *Arch. tisiol.*, May, 1959, 14: 396–406. (*U. of Naples, Italy)

A case of tracheal cyst found after death in a tuberculous patient is described. This rare formation was located at the inferior third of the trachea, between it and the esophagus. The epithelium of the mucosa was of the columnar stratified type, of varying thickness; the cavity of the cyst was connected with the trachea by a very small opening. The importance of such a finding in bronchographic studies is emphasized.

I. ARCHETTI

The Electrophoretic Protein Pattern During Acute Pulmonary Edema (in Italian). V. CORSI*, M. GAGLIO, and O. MAZZONE. *Gior. med. e tisiol.*, January–February, 1959, 8: 27–41. (*U. of Catania, Italy)

The serum protein pattern was studied in 13 cases of acute pulmonary edema by paper electrophoresis using the Grassmann-Hannig technique. Samples of serum were taken during the early phase and about 50 to 60 minutes after the end of the attack.

According to the results obtained during the attack, in comparison with those at the end which were taken as controls: total protein content generally showed slight changes; albumin was almost always (10 cases), and sometimes conspicuously, reduced; the α-1-globulin pattern was not clear; the α-2-globulins were evidently and constantly increased; β-globulins were increased in 5 cases and reduced in 6 cases; γ-globulin variations were not significant.

Such modifications in the serum protein pattern would probably be caused by the characteristic hemodynamic changes of acute edema.

I. ARCHETTI

The Significance of Pleural Effusion Complicating Otherwise Operable Bronchogenic Carcinoma. G. L. BRINKMAN*. *Dis. Chest*, August, 1959, 36: 152–154. (*Henry Ford Hosp., Detroit, Mich.)

The presence of pleural effusion, whether malignant cells are demonstrated in the fluid or not, is of serious prognostic significance. At thoracotomy, 17 of 21 patients showed evidence of extrapulmonary spread of their disease, and only 1 has survived for more than 2 years. It is questioned whether thoracotomy is justified in these patients, even though malignant cells are not demonstrated in the pleural fluid.

E. A. ROUFF

Prognosis in Cases with Eosinophilic Pleural Effusion. K. A. J. JÄRVINEN* and A. KAHANPÄÄ. *Acta med. scandinav.*, June 30, 1959, 164: 245–251. (*Maria Hosp., Helsinki, Finland)

This study was based on 17 cases of eosinophilic pleurisy, either entirely independent or associated with a transitory pulmonary infiltration. These eosinophilic pleurisies accounted for 1.8 per cent of the total series of "primary" exudative pleurisies. The degree of pleural eosinophilia varied from 11 to 70 per cent. The pleural exudate was admixed with blood in 10 cases. Simultaneous eosinophilia of the blood was found in 10 patients. The cure in the cases of eosinophilic pleurisy was generally more rapid, even in the absence of specific therapy, than that usually obtained in idiopathic exudative pleurisy. The average duration of hospitalization was 43 days. To determine the long-term results in patients with eosinophilic pleurisy, follow-up examinations were carried out 5 to 12 years after the onset of the disease. It appeared that 2 patients had developed pulmonary tuberculosis. This morbidity must be considered low compared with cases of idiopathic exudative pleurisy: a control study showed that pulmonary tuberculosis developed in 21 of 52 such cases (approximately 40 per cent). Pleurisy recurred in 2 of the patients with eosinophilic pleurisy. The results of the investigation suggested the following etiologic factors: eosinophilic pleurisy is fairly strongly sex-linked (of the 17 cases, 15 were males); there was an antecedent respiratory infection in 10 cases; 4 patients with eosinophilic pleurisy had a coincidental peptic ulcer; bronchial asthma appeared later in 2 patients.

E. DUNNER

Bullous Emphysema (in German). F. HEINE*. *Beitr. Klin. Tuberk.*, December, 1958, 119: 305–336. (*Med. Univ. Klinik, Münster, Westring 3, Germany)

This disease is described by various names, such as "vanishing lung," "progressive Lungendistrophie," "cotton candy lung," and "Lufteystenerkrankung der Lunge," among others. The pathogenesis of bullous emphysema has several features in common with substantial pulmonary emphysema, and is considered only a special form of the latter. Both develop in older age, very seldom in women, and are characterized by slow progress. Vessel changes are marked in both. The bronchographic pictures are identical; so are the findings in pulmonary function examinations (expiratory stenosis, pathologic Tiffeneau test, and pathologic respiratory quotient).

The morbid anatomy, roentgenography, differential diagnosis, and treatment of the disease are discussed in connection with 5 case histories presented.

Z. VIRÁGH

Tropical Eosinophilic Syndrome. M. GELFAND* and H. BERNBERG. *Central African J. Med.*, August, 1959, 5: 405–411. (*Salisbury European and African Hospitals, Southern Rhodesia, South Africa)

In Southern Rhodesia, various parasites may be associated with the eosinophilic syndrome. These include *Ascaris lumbricoides*, visceral larva migrans (due to *Toxocara canis* and *cati*), bilharziasis (*S. mansoni*), and possibly an animal filarial worm. The exact cause in each case may be impossible to determine and, indeed, eosinophilia in a mild degree without symptoms is a common finding in Rhodesia. In more severe cases (the leukocyte count was 55,000 with 85 per cent eosinophils), biopsy or night blood specimens may reveal filarial parasites. In other instances empirical treatment with cortisone and parasiticidal drugs may be effective despite the failure to demonstrate a specific cause.

E. ROTHSTEIN

Acute Eosinophilic Pneumonia Possibly Due to Infection with *Ascaris Lumbricoides*. I. M. PATZ*. *Central African J. Med.*, August, 1959, 5: 399–404. (*Middelburg, Transvaal, South Africa)

Twelve Bantu in Middelburg, Transvaal, became ill within 24 hours. All had fever (to 104°F.) with general and pulmonary symptoms (exhausting dry cough, wheezing, and dyspnea). The illness lasted about 2 weeks, with complete recovery. Chest films varied from increased markings to diffuse bronchopneumonia; all cleared completely after several weeks to months. Eosinophilia from 7 per cent to 63 per cent was noted in each case, with marked eosinophilia in the sputum. *Ascaris lumbricoides* ova were seen in the stools in 11, and most of the patients later passed large numbers of round worms in the stools; some vomited worms as well. When 2,000 ova of *A. lumbricoides* were swallowed (Koino, 1922) chills, headache, and cough developed on the sixth day, with subsequent dyspnea, wheezing, and continuous coughing, and with larvae in the sputum. Most such infections are slight, but at least one fatality was recently reported in which numerous larvae were demonstrated in the bronchioles.

E. ROTHSTEIN

Report of a Case of Diffuse Interstitial Fibrosis of the Lungs (Hamman-Rich Syndrome) Successfully Controlled by Prednisolone. N. McK. BENNET*. *Med. J. Australia*, July 18, 1959, 2: 84–87. (*Fairfield Hosp., Melbourne, Australia)

A review of the clinical, pathologic, and physiopathologic features of this syndrome precedes the presentation of a case of Hamman-Rich syndrome occurring in a 37-year-old female. A lung biopsy was performed and the diagnosis confirmed by histologic techniques. The clinical course was progressively downhill with marked respiratory distress and impairment of pulmonary function. Prednisolone was started and a rapid improvement in symptoms and signs supervened. The patient was essentially well 9 months after steroid therapy was instituted. The report concludes with a review of the use of steroid therapy in this disease, which suggests that the initial dose of steroids administered should be high.

H. SIMON

Invagination of the Esophagus in Hiatus Hernia. H. Odegaard*. *Acta radiol.*, June, 1959, 51: 443–448. (*Sentralsykehuset, Fredrikstad, Norway)

Retrograde prolapse of redundant gastric mucosa into the lower esophagus may occur in the presence of esophagogastric relaxation. Such prolapse may cause dysphagia, regurgitation, vomiting, and epigastric distress. Another type of invagination associated with hiatal hernia and rarely mentioned in the literature is invagination of the esophagus into the stomach. The condition is not considered common, but this is not indicative of its actual frequency. Of 35 cases of hiatal hernia, 31 show evidence of esophagogastric invagination. The case findings in 8 such cases are given in brief. The invagination is best demonstrated with the patient in the prone position. The question of why and how such invaginations occur has not been solved; but movement of the mucosa downward into the stomach probably creates the condition.

E. SHABART

The Alcohol-intolerance Syndrome in Hodgkin's Disease. J. Bichel*. *Acta med. scandinav.*, June 6, 1959, 164: 105–112. (*U. of Aarhus, Denmark)

The alcohol-intolerance syndrome may not be pathognomonic, but it occurs more frequently in Hodgkin's disease than in any other condition. This syndrome may perhaps be regarded as a curiosity, but it is of both practical and theoretic importance to know it and to subject it to further studies which may contribute to the elucidation of the pathogenesis of Hodgkin's disease.

E. Dunner

Cardiorespiratory Failure in Kyphoscoliosis. E. H. Bergofsky*, G. M. Turino, and A. P. Fishman. *Medicine*, September, 1959, 38: 263–317. (*Columbia U. College of Physicians & Surgeons, N. Y., N. Y.)

The present study was designed to investigate the disorders of respiration and circulation which lead to cardiorespiratory failure in kyphoscoliosis. For this purpose, clinical appraisal was supplemented by anatomic observations and physiologic studies.

The results indicate that cardiorespiratory failure in kyphoscoliosis stems from a combination of alveolar hypoventilation and cor pulmonale. The alveolar hypoventilation is attributable to an unusual pattern of breathing, which operates to minimize the work and energy cost of mobilizing the deformed chest; the cor pulmonale arises from pulmonary arterial hypertension which is attributable, in large part, to the small size of the pulmonary vascular bed.

On the basis of these studies, it has been possible to relate the degree of skeletal deformity to the evolution of cardiorespiratory failure, to define the natural history of the disease, and to outline a therapeutic program based on physiologic principles (Authors' summary).

Erythema Nodosum as a Manifestation of Histoplasmosis. S. Saslaw* and F. M. Beman. *J. A. M. A.*, July 4, 1959, 170: 1178–1179. (*Ohio State U., Columbus)

An 18-year-old college student came from Denmark to Ohio in September, 1958. At that time she had a negative histoplasmin skin reaction. Three months later, she developed erythema nodosum and malaise. A histoplasmin colloidin agglutination test was positive. The histoplasmin skin test gave a strongly positive reaction. The chest roentgenogram showed bilateral adenitis and right middle lobe pneumonia. Recovery was uneventful.

H. Abeles

Pulmonary Histoplasmosis and Its Treatment (in German). T. Wegman*. *Schweiz. Ztschr. Tuberk.*, No. 6, 1958, 15: 469–475. (*Med. Klinik, Kantonspital, St. Gallen, Switzerland)

The case history is presented of a 25-year-old male patient raised in South America. There he was hospitalized first in 1951 with pulmonary infiltration, fever, and erythema nodosum, receiving 30 gm. of streptomycin as treatment. In 1955, his pulmonary condition recurred and 6 months' sanatorium treatment followed, including 166 gm. of streptomycin and 165 gm. of isoniazid. Tuberculin tests were consistently negative and no acid fast bacilli were found in the secretions. In 1958, control examination in St. Gallen, Switzerland, revealed no remarkable clinical symptoms; several calcifications were noted roentgenographically in both lungs; a tuberculin test was negative; however, the histoplasmin skin test was strongly positive, as was the complement-fixation reaction in 1:32 dilution. The apparent good response to antimicrobials is very much in doubt and is, rather, considered a spontaneous healing. The present-day treatment of histoplasmosis is discussed.

Z. Virágh

Pulmonary Alveolar Proteinosis: Report of a Case. D. McDowell*, S. E. Williams, and J. R. Hinds. *Australasian Ann. Med.*, May, 1959, 8: 137–142. (*Green Lane Hosp., Auckland, New Zealand)

A case of pulmonary alveolar proteinosis is reported in the belief that this is the first case to be recognized in New Zealand and, possibly, in Australia. The patient, a Chinese male aged 47, fruiterer by occupation, at the time of examination gave a history of increasing shortness of breath during the previous 3 years with cough and expectoration of mucoid sputum. A chest roentgenogram 2 years previously had shown diminished translucency of both lung bases with fine nodular shadows throughout. His history also included possible infectious hepatitis one year previously, and bilateral basal bronchopneumonia a few weeks prior to admission. On examination he had frequent shallow respirations, gross finger clubbing, and showers of fine crepitations at both lung bases.

He was given a 3-week course of streptomycin plus isoniazid without relief, but no significant antimicrobial or corticosteroid therapy. His clinical condition continued to deteriorate during the following few months, with increasing dyspnea and cyanosis and progressive involvement of lung fields to the point of almost complete obliteration in the roentgenographic picture. Treatment consisted mainly of oxygen inhalations for the relief

of dyspnea and cyanosis and sedatives for cough and insomnia; also, potassium iodide on the slender evidence of possible monilial infection: this was in fact given in massive doses by error for several weeks. About that time, some 3 months after admission, a lung biopsy was performed and he then began to improve rapidly, both clinically and roentgenographically. A year later he was clinically well and a roentgenogram showed almost complete clearing of the lung involvement. Four years later he was still well.

During this patient's stay in hospital all investigations were within relatively normal limits except the lung biopsy, which showed no evidence of interstitial lung disease, but pulmonary alveoli filled with structureless proteinaceous material containing large peripheral histiocytes, which were considered to be identical with the "septal" cells of Rosen, Castleman, and Liebow, reported in their cases of pulmonary plveolar proteinosis.

The report of this case describes a patient who completely recovered from an obscure and life-threatening illness with gross roentgenographic and pathologic changes. It was believed that these findings merited a diagnosis of pulmonary alveolar proteinosis.

R. M. Ford

Pseudo Pectus Excavatum in the Newborn Due to Lower Respiratory Tract Disease. R. L. Clemmens* and A. G. Reyes. *A.M.A. J. Dis. Child.*, June, 1959, 97: 864–867. (*U. of Maryland Hosp., Baltimore)

In a series of 4,216 consecutive newborn infants, 9 developed depression of the lower portion of the sternum after the onset of respiratory distress. Seven of the infants were premature. Two of the babies died; both had respiratory distress and funneling of the lower sternum at the time of death. The anterior chest wall returned to normal by the 11th day in all of the infants who survived. A brief discussion is presented of the mechanism whereby the anteroposterior diameter of the superior portion of the thorax could be increased and, simultaneously, the same measurement in the inferior portion decreased as a result of the same process. It is pointed out that, in pseudo pectus excavatum, depression of the sternum is the result of dyspnea and not the cause of it (Authors' summary).

M. J. Small

Chondrogenous Bronchial Stenosis (in Hungarian). J. Horányi*. *Tuberkulózis*, October–December, 1958, 10–12: 252–258. (*U. of Budapest, Hungary)

Inborn or sustained anomalies of the bronchial cartilage influence the shape and diameter of the bronchial lumen. These irregularities may cause clinical symptoms and, eventually, pathologic changes in the lung. Several case histories illustrated by photomicrographs of bronchi in resected specimens are presented. Among them are those of a 13-year-old boy with situs inversus viscerum. His left middle and lower lobes were removed because of bronchiectasis. Only vestigial cartilage development was found histopathologically in the bronchi.

Z. Virágh

Tietze's Disease of Eight Years' Duration. H. Ausubel*, B. D. Cohen, and J. S. LaDue. *New England J. Med.*, July 23, 1959, 261: 190. (*Cornell U. Med. College, N. Y., N. Y.)

The syndrome of pain and swelling in the region of the costochondral junction may be reproduced by a number of pathologic conditions involving the anterior chest wall. Tietze's disease, however, is idiopathic, arises spontaneously, involving histologically normal cartilage, and follows a fluctuating course with relapses and remissions. A case of Tietze's disease recurring over an 8-year period is reported. The pain produced has been confused with pulmonary infarct, pneumothorax and, most notably, angina pectoris, as in the present case. Treatment of Tietze's disease in the past has been symptomatic and essentially ineffective in the acute phase. Recent reports indicate complete relief after local instillation of hydrocortisone. The response achieved in this patient confirms this finding.

M. J. Small

Serotonin Metabolism in Carcinoid Syndrome with Metastatic Bronchial Adenoma. R. E. Schneckloth*, W. M. McIsaac, and I. H. Page. *J. A. M. A.*, July 4, 1959, 170: 1143–1147. (*Cleveland Clinic Foundation, Ohio)

A 45-year-old man underwent lobectomy for a bronchial adenoma of the carcinoid and adenocystic type. Three months after the operation the patient developed episodes of flushing of the face with nausea, vomiting, sweating, and palpitations. The mean daily excretion of 5-hydroxyindoleacetic acid was found to be approximately 10 times the normal. The urinary excretion of serotonin was unaffected by the administration of two serotonin antagonists. The symptoms increased in frequency and two years after the lobectomy the patient underwent an exploratory laparotomy. The liver was found to be studded with carcinoid tissue. The metastatic tissue contained a hydroxindole, probably an oxidation product of serotonin, in greater amounts than serotonin itself; its existence may

explain variations in the clinical picture of the carcinoid syndrome.

H. ABELES

Tracheo-bronchial and Pulmonary Chondro-adenoma (Hamartoma). D. C. PERRY*. *Brit. M. J.*, June 20, 1959, No. 5137: 1572–1575. (*Royal Shore Hosp. of Sydney, Australia)

Ten cases of chondro-adenoma (hamartoma) are reviewed. Although the majority of these tumors occur in the lung parenchyma, a few occur in the bronchial tree and may present the clinical features of bronchial obstruction. In several instances, increase in the size of the lesion was noted during the adult life of the patient. The pathologic, clinical, and roentgenographic features are discussed.

E. A. RILEY

Pulmonary Granuloma due to Brucellosis. S. J. RAMAH*, B. CHOMET, J. MCLEAN, and C. LEMKE. *J. A. M. A.*, August 1, 1959, 170: 1665–1667. (*V. A. West Side Hosp., Chicago, Ill.)

In a 42-year-old man, an empyema due to hemolytic Streptococcus was followed within several months by the development of a well-defined coin lesion in the left lower lobe. On resection, a granulomatous lesion was found which yielded *Brucella suis* on culture. Cultures for tubercle bacilli and fungi were negative.

H. ABELES

Electrocardiographic Changes in Neoplasms. I. DOSKOV, E. PETKOV, and L. PETKOVA. *Abstracts of Bulgarian Scientific Lit.*, No. 2, 1959: 28 (abstracted from *Sŭvr. Med.*, No. 11–12, 1958, 9: 77–84 (in Russian)).

Electrocardiographic studies are reported of 30 patients aged 30 to 55 suffering from neoplasms of different localization but with no histories and clinical data of cardiac disorder. The neoplastic disorder dated back 5 months in 11, and more than 6 months in 19 of the patients. Sinus tachycardia was established in 17, most of them with advanced neoplasms, and sinus bradycardia in only 2. The following impairments of conduction were observed: a prolonged atrioventricular time in 2, intraventricular disorders in 5, and right arborization block and general data for conduction impairment in 6 patients. These changes are observed most commonly in advanced neoplastic disorders. There was evidence of impairment of the right half of the circulation and depression of ST segments in 18 patients, 17 of whom were in advanced stages. Patients suffering from neoplasms with no metastases and duration of the disorder less than 6 months had normal electrocardiograms (with the exception of 1 patient with a focal myocardial lesion).

In general, it is established that electrocardiographic evidence of diffuse myocardial lesions is very common in advanced neoplastic disorders, and is not encountered in the early stages of the disease. The causes for myocardial changes in malignant disease are the direct effect of the toxic products of neoplasmatic and myxopaemic degeneration due to accompanying anemia, with toxicity playing a predominant role (after original abstract).

Sarcoma of the Trachea (in French). H. ROMANOFF* and H. MILWIDSKY. *J. franc. med. et chir. thorac.*, August 28, 1958, 13: 267–272. (*Hadassah U. Hosp., Jerusalem, Israel)

This is the case of a 48-year-old woman who developed the following symptoms over a period of one year: she complained of cough with expectoration, occasionally blood-streaked sputum, and pain on swallowing; on occasion, especially when swallowing, she felt that she was suffocating. She was admitted to the hospital and bronchoscopy and roentgenograms of the chest revealed a tumor of the trachea. On biopsy the diagnosis of sarcoma was made and the tumor was removed surgically with good results. A primary malignant tumor of the trachea, like the one described, is a rare occurrence.

E. LYON

TUBERCULOSIS, PULMONARY

Should the Term "Minimal" Pulmonary Tuberculosis Be Used? (in French). S. MONTANI*. *Schweiz. Ztschr. Tuberk.*, No. 6, 1958, 15: 475–496. (*Path. Institut, Kantonspital, Lucerne, Switzerland)

A diagnosis of "minimal" pulmonary tuberculosis is not precise. A group of French phthisiologists denote a pulmonary tuberculosis process as "minimal" if the lesion is not larger than 2 cm. on each side, shows only minimal opacity roentgenographically, and clinical symptoms are absent. Several authors accept the definition of the American National Tuberculosis Association; others in the various countries again have different standards concerning the term.

Of 500 patients of the Sanatorium Valaisan, only 40 were admitted with the diagnosis of "minimal" tuberculosis, and the conditions of some of them worsened even under sanatorium care. Another 40 patients with "minimal" tuberculosis were admitted to institutional care too late. Of 312 patients in 3 tuberculosis outpatient clinics, 44 per cent were diagnosed as having "minimal" disease; 33 per cent of these developed advanced tuberculosis.

"Minimal" pulmonary tuberculosis is not a special form of tuberculosis; consequently, the term is misleading. Home treatment of this condition is not advisable.

Z. Virágh

Bronchoscopic Findings in Atelectasis (in German). H. Guntert*. *Schweiz. Ztschr. Tuberk.*, No. 6, 1958, 15: 497–510. (*St. Claraspital, Basel, Switzerland)

Among 3,000 patients, atelectasis was detected roentgenographically in 140, of whom 100 were also studied by bronchoscopy. Total bronchial stenosis was found in 25 patients, high degree obstruction in 29, while 46 patients had normal or only slightly obstructed bronchial lumens; consequently, in these latter cases the development of atelectasis could not be explained. The most frequent cause of bronchial obstruction was endobronchial tuberculosis (41); tuberculous adenopathy and tumor were involved in 3 cases each.

Z. Virágh

Studies on Late Functional Cardiorespiratory Repercussions Caused by Surgical Collapse Therapy in Chronic Pulmonary Tuberculosis (in Italian). M. Caracciolo*, G. Cardaci, P. G. Ramacciotti, and G. DeRitis. *Gior. med. e tisiol.*, March–April, 1959, 8: 183–286. (*Istituto C. Forlanini, Rome, Italy)

This series of 11 papers, written by the above authors with a group of 5 other co-workers, describe the studies on the behavior of the cardiorespiratory function in patients with chronic pulmonary tuberculosis who were treated with thoracoplasty or with extrapleural pneumothorax, and examined 2, 4, or more years after surgery.

The main conclusions are: (*1*) Thoracograms were better in the young than in the aged patients, and thoracodiaphragmatic function was better in those who had extrapleural pneumothorax rather than thoracoplasty. (*2*) Functional results were more favorable in patients with recent lesions. (*3*) Oxygen consumption was slightly decreased in young patients who had upper thoracoplasties or normal extrapleural pneumothorax, and much more diminished in those who had large thoracoplasties or pneumothorax with empyema; the ventilation coefficient, i.e., the ratio between ventilation and oxygen consumption was, therefore, increased; the arterial oximetry was slightly below normal values. (*4*) The respiratory dead space was normal in young patients who had upper thoracoplasties and normal extrapleural pneumothorax; it was increased in aged patients who had subtotal thoracoplasties or extrapleural pneumothorax with empyema; the same behavior was found for residual volume, for the ratio between alveolar and pulmonary ventilation, and for alveolar air composition. (*5*) Damage to the cardiac muscle was found by electrocardiography in 35.6 per cent of the patients who had thoracoplasties, and in 22.9 per cent of those who had extrapleural pneumothorax; the arterial pressure and the cardiac rhythm were normal. (*6*) The systolic, diastolic, and mean pressures of the right auricle and ventricle and pulmonary artery were almost normal in patients who had upper thoracoplasties and extrapleural pneumothorax, while these values were increased in patients who had large thoracoplasties. (*7*) Bronchospirometry showed good functional capacity of the re-expanded lung in patients who underwent extrapleural pneumothorax, while it was very reduced in those who had large thoracoplasties. (*8*) A comparative study of the respiratory functional capacity, as revealed by various tests, showed that it was better in patients who underwent extrapleural pneumothorax rather than thoracoplasty. (*9*) The vital capacity and maximal direct ventilation values were little reduced in patients who had upper thoracoplasties and normal extrapleural pneumothorax, and were obviously diminished in those who underwent subtotal thoracoplasty and those patients who had pneumothorax with empyema. (*10*) The cardiorespiratory function in patients 8 years or more post surgery was much better in those treated with extrapleural pneumothorax without complications than in those who had thoracoplasties. (*11*) Cardiorespiratory function was studied before and after surgical treatment and its picture in the following years is given.

I. Archetti

Tuberculosis in Childhood. Part III. Regressive Pulmonary Infiltrates of Tuberculous Origin ("Epituberculosis"). P. Schwartz*. *Arch. Pediat.*, July, 1959, 76: 255–268. (*Warren State Hosp., Pennsylvania)

Under certain conditions avirulent germs may induce extensive, even lobar, necroses and disintegration; that is, changes which we traditionally consider as signifying aggravation of the phthisis and an unfavorable prognosis, although they are characterized in these cases by a predominant disposition for healing. On the other hand, virulent tubercle bacilli given intravenously or intratracheally after an interval following a preparatory treatment may behave as avirulent agents; that is, they induce a disease which heals after reaching, in a relatively short time subsequent to the superinfection, the maximum of its extension and the highest degree of its destructive capabilities.

It is concluded that—at least in animal experiments—necroses, softening processes, and cavita-

tion in general, even if very extensive, signify not a malignancy of the disease, but the effect of relatively large quantities of tubercle bacilli and their substances, or the presence of a high susceptibility to inflammatory reactions. Similarly, the manifest healing tendency of a tuberculous lesion does not prove definitely the benignity of the process, but can be considered only as an indication of the fact that effective immunity is present.

M. J. SMALL

Tuberculosis in Childhood. Part IV. Regressive Pulmonary Infiltrates of Tuberculous Origin ("Epituberculosis"): Morphology and Pathogenesis of the Reversible Inflammatory Infiltrations in Human Pathology. P. SCHWARTZ*. *Arch. Pediat.*, August, 1959, 76: 299-321. (*Warren State Hosp., Pennsylvania)

In the pathogenesis of predominantly caseous lymphonodogenic aspiration-infiltrations, the quantity of the discharged necrotic and disintegrated lymph node masses evidently is of decisive importance. Voluminous lymphonodogenic bronchial defects were present, concordant with the participation of excessively large lymph node complexes. We deal in these cases with similar occurrences as in experiments in which pre-infected animals received particularly large doses of an intratracheal superinfection; by a massive lymph node discharge into the bronchial system, a pre-inflammatory prestasic circulatory stagnation, involving an extensive pulmonary area, was subjected to a definitive or prolonged interruption.

M. J. SMALL

The Use of Prednisone in Primary Tuberculosis in Children. K. W. LAVERS* and J. C. ROBERTS. *Tubercle*, June, 1959, 40: 173-176. (*Harefield Hosp., Middlesex, England)

From a large group of cases of primary pulmonary tuberculosis admitted to hospital, a pilot study was carried out on 10 children whose disease either showed no change or became worse after a period of chemotherapy. Prednisone was administered in addition to the antituberculosis drugs in an endeavor to determine whether the progress of the disease was influenced. In at least 7 of the children, prednisone appeared to influence the lesions favorably. On the whole, the improvement which occurred with prednisone therapy was neither rapid nor dramatic. This may in part be due to the small doses employed. In no case did deterioration of a lesion occur while prednisone was given, and in no case was it thought that any ill effect had resulted from the use of the steroid.

M. J. SMALL

How Chronic is Pulmonary Tuberculosis in the Elderly? J. SMITH*. *Brit. M. J.*, June 6, 1959, No. 5135: 1448-1451. (*Ruchill Hosp., Glasgow, Scotland)

The previous roentgenographic histories of 100 consecutive cases of pulmonary tuberculosis in which the patients were older than 55 years were investigated. Fifty had been filmed before: 23 (46 per cent) of these films were clear and 27 (54 per cent) showed an abnormality. In films considered abnormal, inactive tuberculosis, "unresolved pneumonia," and "bronchitic changes" were the most common abnormalities found. Among those 50 cases, only 3 were below the age of 50 when the original film was taken. Seventy-six per cent of the patients had been filmed within 5 years of diagnosis. The high percentage of negative chest films within a short time prior to the development of active tuberculosis suggests that the disease in the elderly person may not always be chronic and of long standing duration.

E. A. RILEY

Acute Tuberculous Empyema Cases Followed Up with Antistaphylolysin (ASta) and Antistreptolysin (AS) Titers. A. WESTERGREN*. *Acta med. scandinav.*, June 30, 1959, 164: 185-201. (*St. Gorans Sjukhus, Stockholm, Sweden)

Of 181 cases of acute tuberculous empyema, 50 showed yellow staphylococci in the empyema effusion; in 20 others, there was mixed infection without yellow staphylococci; and in the remaining 111, no mixed infection was demonstrated. The antistaphylolysin (ASta) titer showed a rise after the onset of empyema in 96 per cent of those cases in which staphylococci had been found, and in 38 per cent of all of the other cases. In typical cases, this elevation commenced after about 2 weeks, and reached its maximum (in 46 per cent of the cases, 8.0 units or more), after a further 2 or 3 weeks. The later course of the titer seemed, on the whole, to be fairly well correlated to improvement or worsening of the empyema.

In view of the ASta curve, et cetera, it is probable that staphylococci were also implicated in the empyema in approximately ½ of those cases in which ASta elevations, but not positive findings of staphylococci, were recorded. In a few cases, a corresponding relation between the antistreptolysin (AS) titer and findings of B-hemolytic streptococci could be demonstrated, but only 25 of the 181 cases exhibited AS titer rises in connection with the empyema and, with few exceptions, the AS curve presented a much weaker correlation to the course of empyema than did the ASta titer. The general behavior of the AS titer in this material, however, may be emphasized as illustrating the interplay of nonspecific infections, frequently

without noteworthy direct clinical symptoms. The two titers showed parallel rises and falls in a small number of cases but, on the whole, they ran quite independent courses.

Tuberculous patients often show a tendency to high titers, but the titer elevations should invariably be interpreted as a response of the organism to yellow staphylococci and B-streptococci, respectively. The staphylococcic pleural process as a rule must come about through activation and spread of latent foci, chiefly in the lymph nodes.

E. DUNNER

Examination of the Endometrium in Patients with Active Pulmonary Tuberculosis (in German). B. SZENDI* and I. LAKATOS. *Beitr. Klin. Tuberk.*, January, 1959, 119: 434–438. (Vármegyei Közkórház, Gyula, Hungary)

The endometria of 100 patients with active pulmonary tuberculosis were examined histologically and bacteriologically; 4 cases of genital tuberculosis were diagnosed. In 2 additional cases, *M. tuberculosis* was cultured from the uterus mucosa, but no disease was found histologically. Pulmonary tuberculosis has no effect on the reproductive ability of the body. Parturition occurred in 89 patients; the 4 patients with genital tuberculosis did not become pregnant.

Z. VIRÁGH

Glucocorticoids in the Treatment of Pulmonary Tuberculosis and Serous Pleural Effusion (in German). F. HEINE* and E. SCHÜRMEYER. *Beitr. Klin. Tuberk.*, February, 1959, 119: 516–530. (*Universitätsklinik, Münster, Germany)

Prednisone was administered to 15 patients with pulmonary tuberculosis whose disease did not respond to conventional antimicrobials after 8 weeks. In only 1 case was improvement noted. All but 2 of 16 patients with serous pleural effusions improved remarkably when prednisolone, another steroid, was administered intrapleurally. The instillations were done when no response was observed after 3 weeks of antimicrobial therapy.

Z. VIRÁGH

Asian Influenza and Pulmonary Tuberculosis. S. LÖFGREN* and A. CALLANS. *Acta med. scandinav.*, August 31, 1959, 164: 523–527. (*St. Görans Hosp., Stockholm, Sweden)

Among 204 patients admitted for progression of pulmonary tuberculosis between October 1, 1957 and March 31, 1958, there were at least 28 (13.7 per cent) and probably 42 (20.3 per cent) in whom the progression of tuberculosis had directly followed an attack of Asian influenza. The findings indicate the importance of special care in the follow-up of patients with tuberculosis following influenza or other intercurrent infections. Similar observations were made during the 1918 influenza epidemic.

E. DUNNER

Pneumonia in Pulmonary Tuberculosis (in German). W. BLUMENTHAL*. *Beitr. Klin. Tuberk.*, February, 1959, 119: 481–498. (*Berlin-Buch, Karower str. 11, Germany)

There is no known criterion that may definitely establish the diagnosis of pneumonia in patients with pulmonary tuberculosis. When a tuberculous patient falls ill, showing the characteristic signs and symptoms of pneumonia, a nontuberculous etiology is most likely. This statement refers not only to the classical picture of lobar pneumonia, but to all forms of pulmonary inflammatory processes known as pneumonias. When the elevated temperature returns to normal rather quickly following the administration of penicillin alone or in combination with sulfonamides, it makes the nontuberculous origin of the disease likely; speedy regression roentgenographically ascertains it. Keeping in mind the grave prognosis of pneumonia in cases of pulmonary tuberculosis, these patients should first be treated for pneumonia. The so-called "mixed infections" are more frequent than is generally believed.

The case histories of 11 patients are presented as illustration.

Z. VIRÁGH

Vascular Thrombosis in Tuberculous Patients Treated With Steroids (in French). L. A. GERVASONI*. *Schweiz. med. Wchnschr.*, July 4, 1959, 89: 713–719. (*Clinique Sylvana, Lausanne, Switzerland)

In 1,667 patients receiving antimycobacterials alone, 11 thromboembolic accidents were seen (0.6 per cent), whereas 8 (6 per cent) were seen among the 133 patients who received antimycobacterials with corticoids: cortisone, hydrocortisone, prednisone, or corticotropin. In 20 patients of each group a study with Hartert's thromboelastograph and the platelet count was made, and the prothrombin index was determined. Under the effect of steroids a state of hypercoagulability was produced, with a return to normal values a few days after the interruption of the treatment. This phenomenon was not seen in the group not receiving steroids. A distinction is made between purely inflammatory phenomena (phlebitis) and thromboembolic complications, which occur most frequently in patients with a past history of vascular troubles.

J. HAAPANEN

Frequency and Clinical and Roentgenographic Features of Tuberculous Localization in the Upper Axillary Segments of the Lung (in Italian). V. Gammarrota*, G. De Ritis, and G. Pallotta. *Ann. Ist. Carlo Forlanini*, No. 3, 1958, 18: 266–294. (*Istituto C. Forlanini, Rome, Italy)

Patients with localization in the upper axillary segments of the lung were selected among 2,908 hospitalized patients with clear zonal tuberculosis: the incidence was 8.3 per cent in a group of 1,182 cases observed, particularly in young males (19 to 36 years old), before antimicrobials were used; and 11.8 per cent in a group of 1,726 cases found thereafter, with an evident prevalence among males (23 to 50 years old). The percentages of different forms of tuberculosis in the two groups were as follows: infiltrates (4.1 and 3); miliary dissemination (14.2 and 15.2); cavitary lesions (73.5 and 54.4); tuberculomas (8.2 and 27.4).

The clinical and roentgenographic features of these localizations were very similar to those observed in lesions of other segments of the lung. The heavier respiratory trauma of the upper segments, however, calls for early medical or surgical collapse therapy together with the use of antimicrobials.

I. Archetti

TUBERCULOSIS, NONPULMONARY

Extrapulmonary Tuberculosis in Repatriated Prisoners of War (in German). F. E. Räntsch*. *Tuberkulosearzt*, June, 1959, 13: 400–409. (*Steinmetzstr. 1, Neumünster, Germany)

The incidence of extrapulmonary tuberculosis in 1,283 ex-prisoners of war was 17.8 per cent, with an incidence of 23.4 per cent in those who had been repatriated shortly after the end of the war; it was 13.4 per cent in late repatriates. This difference might depend on 2 factors: selection, due to conditions in the camps; and higher incidence of late primary infections in the early repatriates. The incidence of pleurisy prior to the development of extrapulmonary tuberculosis was higher in ex-prisoners of war (46.9 per cent) than in civilians, but there was no difference between the 2 groups with regard to the combination of pulmonary and extrapulmonary tuberculosis (approximately 20.6 per cent). The lesions were located in the bony skeleton in the majority of cases (56.2 per cent), and in the urogenital tract in 23.7 per cent. Multiple skeletal foci were present in 24.7 per cent of the cases. Tuberculous spondylitis was the most common skeletal lesion (44.3 per cent), the lumbar vertebrae being the preferential site.

J. Haapanen

Tuberculosis of the Heart (in German). P. Richter*, *Beitr. Klin. Tuberk.*, December, 1958, 119: 298–304. (*Kiel, Dreiecksplatz 5, West Germany)

Tuberculosis of the heart is not so rare a disease as may appear from the German literature. The various non-German statistics show an incidence of 0.28 to 4 per cent among tuberculous patients.

Diagnosis is rather difficult; sometimes there are no symptoms at all, or only insignificant clinical signs; in rare cases infarction or rupture develops. The heart tuberculosis is always secondary. Whenever cardiac symptoms suddenly develop in the course of tuberculosis, one should think of their possible tuberculous origin.

The case histories of 2 patients are presented. One died from miliary tuberculosis, and autopsy revealed a tuberculous ulcer in the anterior wall of the heart as well as generalized tuberculosis. The other case was diagnosed by roentgenogram (infiltration in the right lower field, pleural and pericardial effusion) and electrocardiographic changes. Both returned to normal after intensive antituberculosis chemotherapy.

Z. Virágh

Macronodular Tuberculosis of the Liver (in French). R. Joseph, Ch. Nezeloff, J.-C. Job, and A. Wolf. *Semaine hôp.*, June–July, 1959, 35: 342–345.

A 15-year-old girl from southern Algeria was hospitalized for hepatomegaly. The liver was found to be enlarged, hard, and nodular as in neoplastic metastasis. A tuberculin skin test was positive. There was a high sedimentation rate, disturbed flocculation reactions, and beta- and gamma- hyperglobulinemia. An exploratory laparotomy revealed the presence of numerous tubercles within the hepatic parenchyma. The patient was treated with streptomycin and isoniazid and the size of the liver returned to normal.

A review of the literature showed that 23 cases of hepatomegaly due to tuberculosis have been observed and the observations published. The diagnosis can be established only by exploratory laparotomy.

E. Lyon

Penile Tuberculosis (in Hungarian). Z. Szendrői* and A. Csernus. *Tuberkulózis*, October–December, 1958, 10-12: 281-283. (*U. of Budapest, Hungary)

Case histories are presented of 3 patients with tuberculosis of the penis. The diagnosis was confirmed by histopathology of biopsied specimens. Two of the patients also had pulmonary tuberculosis. Antituberculous chemotherapy was employed with good results.

Z. Virágh

Observations on the Diagnosis and Treatment of Genital Tuberculosis (in German). H. May* and I. Strauss. *Deutsche med. Wchnschr.*, May 15, 1959, 84: 944–948. (*Kreuth b. Tegernsee/Obb, Germany)

Isolated Tuberculosis of the Spleen (in German). H. Hofman*. *Beitr. Klin. Tuberk.*, January, 1959, 119: 446–453. (*Krankenhaus Borna b. Leipzig, East Germany)

The case history is presented of a 16-year-old student who was admitted to the St. Georg Hospital, Leipzig, East Germany, with splenomegaly and high fever. Other findings were not contributory, and the Mantoux test (1:10,000) was negative. With a tentative diagnosis of tuberculosis of the spleen, treatment with streptomycin and isoniazid was started but, after about 5 weeks of chemotherapy, splenectomy was performed as the patient's condition worsened.

Histology confirmed the diagnosis of tuberculosis. Tubercle bacilli were not demonstrated. Further examinations did not reveal tuberculosis in any other organ. Continued chemotherapy resulted in complete healing.

Z. Virágh

Spondyloarthritis Ankylopoietica Associated with Tuberculous Spondylitis (in German). W. Pfluger*. *Tuberkulosearzt*, June, 1959, 13: 414–417. (*Sanatoriumstr. 2, Wien 14, Austria)

A case of spondyloarthritis ankylopoietica is described which was complicated by tuberculous disease of the vertebrae. The combination of these two diseases is extremely rare, and there are certain difficulties in the diagnosis of a degenerative inflammatory process in the presence of ankylosis of the vertebral column.

J. Haapanen

"B.C.G. Sarcoidosis." P. Ellman* and L. G. Andrews. *Brit. M. J.*, June 6, 1959, No. 5135: 1433–1435. (*Plaistow Hosp., London, England)

A histologically proved case of sarcoidosis occurred in a girl given BCG vaccination 7 years previously. She recovered spontaneously, and the tuberculin sensitivity, which became negative during her illness, reverted to positive. The literature on the occurrence of sarcoidosis in BCG-vaccinated cases is reviewed. It is suggested that, had she not been vaccinated, she might have developed tuberculosis instead of sarcoidosis. The time interval was too long for vaccination to be regarded as the cause of the sarcoidosis (Authors' summary).

E. A. Riley

THORACIC SURGERY

Treating Lung Cancer. Special Article. J. R. Bignall*. *Tubercle*, June, 1959, 40: 205–222. (*Brompton Hosp., London, England)

This paper reviews the more important published facts about the present treatment of lung cancer. The results of treatment both in prolonging life and relieving suffering are bad. Nor is there at present any hope of immediate improvement. It seems probable that earlier diagnosis would provide more potentially curable cases. But if, as is unlikely, both the proportion who could have a resection and the survival rate after resection were doubled—giving 50 per cent resections and a 50 per cent 5-year survival rate—75 per cent of patients developing lung cancer in 1959 would still be dead by the end of 1964. Greater diagnostic efficiency and improvement of surgical and radiation therapy techniques are, of course, desirable and to be encouraged. But it seems likely that no major improvement in the results of treatment will appear until some method is found of bringing uncontrolled "cancerous" growth back under control.

As about 95 per cent of patients with lung cancers die of the disease within 5 years, the care of the dying may be said to be, quantitatively, the most important part of treatment. Yet it is by far the least publicized and least well investigated. Much effort and expense are put into attempts to prolong life, if only by a few weeks; primary tumors are irradiated when secondaries are almost certainly present, at times depriving the patient of a month's work and wages or of relatively placid life at home unspoiled by daily reminders that he has a diseased lung; and patients with obviously widespread neoplasms are given nitrogen mustard when there is no evidence whatsoever that it is capable of eradicating even a single nodule of lung cancer. But relatively little seems to be being done to investigate, by collection of facts, analysis and classification, or by purposeful trial, the large problem of how best to help the patient with lung cancer to die.

M. J. Small

Pathological Factors in the Prognosis of Bronchial Carcinoma Following Surgical Excision. W. J. Hanbury*. *Tubercle*, August, 1959, 40: 257–264. (*St. Bartholomew's Hosp., London, England)

This is a study of certain pathologic features in 26 patients with 5-year survivals following surgical resection of the lung for bronchial carcinoma. The findings in this group were compared with those in 55 patients operated on during the same period who did not survive 5 years. The purpose was to assess the factors which might influence the postoperative prognosis. The main conclusions reached

were: (*1*) the left upper lobe showed the highest 5-year survival rate in this series; (*2*) the average tumor size in both groups showed little difference and therefore could not have been an important prognostic factor by itself; (*3*) neoplastic involvement of regional lymph nodes was significantly different, occurring in 42 per cent of cases in the 5-year survival group as opposed to 76 per cent of the fatal cases. No paratracheal nodes were found involved among the survivors, compared with 18 per cent in the nonsurvivors; (*4*) squamous cell carcinomas accounted for 92 per cent of the tumors in the 5-year survival group compared with 65 per cent in the nonsurvivors.

Neoplastic infiltration of adjacent structures other than lymph nodes was found in 15 per cent of the 5-year survival cases and in 31 per cent of the fatal cases.

M. J. Small

Considerations on the Indications and Results of 220 Cases of Exeresis in Pulmonary Tuberculosis (in Italian). P. Zorzoli*, A. Orlandoni, and C. Voglino. *Gior. ital. tuberc.*, May–June, 1959, 13: 149–162. (*U. of Pavia, Italy)

In Italy, pulmonary exeresis in cases of tuberculosis is still a relatively rare procedure. The opinions expressed in this paper are based upon 220 operations in patients with pulmonary tuberculosis, and are especially intended to make this therapeutic method more familiar to Italian phthisiologists and surgeons.

The patients generally came late to the surgeon, had been previously treated with many antimicrobials, and 64.5 per cent had been already subjected to various forms of collapse therapy. The mortality rate was 10 per cent: death was more frequent among patients who had had *pneumectomy*, and occurred less among patients who underwent lobectomy. No mortality occurred among the patients who had segmental resection.

The necessity of surgery in early stages of this disease is emphasized, if better results are to be expected. No prophylactic resection should be performed in resting or inactivated cases.

I. Archetti

Mediastinoscopy (in Finnish). A. J. Seppälä*. *Duodecim*, June, 1959, 75: 435–437. (*Kivelän sairaala, Helsinki, Finland)

A new method for mediastinoscopy is described. During intubation narcosis the index finger is pushed downward on the anterior surface of the trachea through an incision in the jugular region. A tube, like a small esophagoscope, is conducted through this canal down to the lymph nodes in the bifurcation and in the tracheobronchial angles. Biopsy of all of these lymph nodes can be carried out and the site of the specimen taken can be marked with a small silver clasp. Altogether, 32 mediastinoscopies were performed without any complications. Among the first 11 cases, 6 cancers, 2 cases of tuberculosis, and one of Hodgkin's disease were diagnosed. In 2 cases no pathologic changes were noticed. In none of these cases was diagnosis made before the procedure was performed.

J. Haapanen

Solitary Pulmonary Nodule. G. H. C. Joynt* and K. P. Vassal. *Canad. M.A.J.*, July 15, 1959, 81: 78–82. (*Toronto Western Hosp., Canada)

Among 60 consecutive solitary nodules resected between 1948 to 1958, 28 (47 per cent) were found to be primary carcinoma of the lung, 5 (8 per cent) metastatic carcinoma, 22 (37 per cent) granulomas, 4 (7 per cent) benign tumors, and 1 was found to be a loculated hematoma. Ten cases of primary neoplasm were treated by pneumonectomy, 17 by lobectomy, and 1 by segmental resection. Ten of these cancers were anaplastic or undifferentiated, 8 were epidermoid or squamous, 8 were adenocarcinomas, and 2 were alveolar cell carcinomas. Nine of these neoplasms occurred in females (32 per cent), 4 of whom had adenocarcinomas and 2, alveolar cell carcinomas. Among these 28 patients, 10 died within 2 years.

Among the 22 granulomas, there was no definite proved lesion caused by a fungus. Cultures for tubercle bacilli and guinea pig inoculation were carried out in 13 cases and were positive in 6. Of the 5 metastatic tumors, 3 originated from a hypernephroma, 1 from an osteogenic sarcoma, and 1 from a melanotic sarcoma. The latter patient is alive and well 3 years after resection. The patient with a metastatic osteogenic sarcoma lived more than 3 years after operation. Of the 4 benign tumors, there were 2 adenomas, 1 harmartoma, and 1 case of localized lymph node hyperplasia. There were no surgical deaths or serious complications in the entire series.

E. A. Riley

Concerning the Technique of Photothoracoscopy (in German). H. Hatzenberger*. *Wien. klin. Wchnschr.*, June 5, 1959, 71: 407–408. (*Krankenhaus der Stadt Wien—Lainz, Vienna, Austria)

A combination of a thoracoscope with a photographic apparatus is described which makes endothoracic color photography possible. The technical problems of this method are not yet solved completely and routine use of this apparatus is not yet possible.

G. C. Leiner

Traumatic Pneumothorax. D. I. Roberts*. *Med. J. Australia*, July 18, 1959, 2: 71–73. (*Launceston, Australia)

The author reviews a personal series of 6 instances of traumatic pneumothorax. Air entering a pleural cavity results in pneumothorax and collapse of the lung unless the lung is held in place by adhesions. Traumatic pneumothorax can result from perforating wounds of the chest wall or from closed injuries. Symptoms usually depend upon the extent of pulmonary collapse and mediastinal shift, and upon the rapidity with which these changes occur. Complications of traumatic pneumothorax are usually considered to consist of tension pneumothorax (when a valvular wound of the lung or chest wall permits one-way entrance of air into the chest cavity), hemorrhage from intercostal vessels or ribs, and sucking wounds. As the intrapleural pressure rises, the mediastinum is pushed to the opposite side, venous return is impeded, and shock ensues.

The diagnosis is often made accidentally in the course of a roentgenographic search for trauma to the thoracic cage. The history may be suggestive, but is rarely diagnostic, and the physical findings may be minimal.

Treatment consisted of closure of open wounds, anchoring the flail chest when present, local anesthesia for pain, oxygen in the presence of cyanosis, and underwater drainage following needle puncture. The author believes that underwater drainage encourages more rapid resorption of trapped air and consequent re-expansion of the collapsed lung tissue. He also believes that drainage may lessen the incidence and severity of late pulmonary complications. The disadvantages of the procedure—hemorrhage and infection—can be avoided by careful attention to technique. Twenty-four hours of drainage was usually sufficient, but stubborn cases required a longer period, often with the addition of 15 cm. of water-negative pressure.

Complications consisted of pleural effusions, hemothorax, empyema, and distant injuries incurred at the time of the original trauma. Pleural effusions developed late and were only aspirated if they were symptomatic. Hemothorax should be aspirated dry. Empyema and other injuries should be treated by accepted principles. Two of the 5 patients developed shock, 1 had hemoptysis, 1 had a flail chest, and 1 experienced a coronary occlusion. The dangers of a traumatic pneumothorax can occur early or late. The early causes of death are shock and anoxia resulting from bleeding, tension pneumothorax, and mediastinal flap. The late danger is that of restricted pulmonary function due to fibrosis and bronchiectasis. These can be avoided by early re-expansion.

H. Simon

Tuberculosis of the Bronchial Stump after Pulmonary Resection (in German). M. Böszörményi*, I. Kertes, O. Schweiger, and I. Ungár *Beitr. Klin. Tuberk.*, January, 1959, 119: 397–406. (*Orsz. "Korányi" Tbc. Intézet, Budapest XII, Hungary)

Recidivation after pulmonary resection may be caused by pathologic changes in the bronchial stump. This "stump bronchitis" may present in the form of an inflammation of the mucosa around the suture, with symptoms of cough, sometimes hemoptysis and, eventually, positive acid-fast bacillary findings. Endoscopic removal of the irritating suture may solve the problem. Another cause could be "suture granuloma"—the term is descriptive—which is often difficult to treat. The third form of stump bronchitis may develop from the small diverticulum created by the surgical closure of the bronchus. This condition can be detected by selective bronchography of the resected bronchus; among 1,400 resections there were 7 cases of this disorder.

Since the stump bronchitis may progress to recidivation of the tuberculous process, it is important to prevent development of this condition. To accomplish this, it is necessary that the amputation of the bronchus be done in healthy tissue; the foreign body effect of the suture should be reduced by modification of the suturing technique; the development of diverticulum should be avoided (Sweet's bronchus-closing method is recommended). Bronchoscopy with eventual removal of the sutures might be indicated. Chemotherapy should continue postoperatively.

Z. Virágh

Indications for and Results of Pulmonary Resection for Tuberculosis (in German). J. K. Kraan* and L. D. Eerland. *Beitr. Klin. Tuberk.*, February, 1959, 119: 499–515. (*Sanatorium "Beatrix-oord," Appelscha, The Netherlands)

In the Thorax Center, Groningen, Holland, 1,500 pulmonary resections were performed for tuberculosis during the period 1943–1958. Resection was considered in those patients in whom the possibility prevailed that the most advanced lesion could be removed with minimal loss in pulmonary function at a relatively small risk. In these patients, resection was performed: (*1*) if the lesion did not change after bed rest and chemotherapy for 7 to 10 months; (*2*) in cases of positive secretions with imminent or manifest resistance to antimicrobials. It can be stated that 70 per cent of the patients discharged from tuberculosis institutions received only bed rest and drug treatment.

The results of resection were tabulated in 1,423 patients through January 1, 1958. Complete healing was achieved in 1,112 patients (89.6 per cent); 130 patients (10.4 per cent) were still under treat-

ment; 1,212 patients (97.6 per cent) became negative for acid-fast bacilli, while 30 patients (2.4 per cent) remained positive. There were 74 deaths, 27 of which occurred in the first 2 months postoperatively. Of the rest, 33 died from nontuberculous causes.

Reactivation was the result of short-term chemotherapy or insufficient dosage. In 3 patients, relapse occurred after pneumonectomy because of considerable lesions in the other lung; another 3 patients relapsed due to infection *per contiguitatem* since the operation was difficult and the healthy lung tissue broke. In several patients who relapsed, the bacilli were of decreased susceptibility to antimicrobials.

Complications included severe postoperative bleeding (2 patients died from hemorrhagic shock); ulceration of the bronchial stump developed in 42 cases; empyema and bronchial fistula were treated in 58 patients. Recidivation occurred in 74 patients. Resection had to be performed again in 62 cases. In 66 patients, bilateral resection was performed. Thoracoplasty before resection was done in 50 cases; after resection, in 38 cases.

Z. Virágh

Changes in Lung Thorax Compliance During Orthopedic Surgery. S. Lynch*, L. Brand, and A. Levy. *Anesthesiology*, May–June, 1959, 20: 278–282. (*Columbia U., N. Y., N. Y.)

A method of study was undertaken in an effort to clarify the cause of adverse effects on the mechanism of breathing with an anesthetized patient in the prone position. Fifteen unselected cases undergoing spinal fusion for the correction of scoliotic deformities, for the stabilization of the spinal column, or for removal of a herniated nucleus pulposus were used in this study. The method is described in detail and a diagram of a simple method of measuring compliance during anesthesia is shown.

Measurements of compliance in the supine position prior to surgery in patients without deformities were in agreement with those of previous investigators. A severe reduction of compliance, below the normal values for anesthetized supine patients, was found in patients with scolioses. A further reduction of compliance was observed in all patients who were placed in the prone position before surgery. Scoliotic patients showed a severe reduction of compliance in the prone position. Low total respiratory compliance persisted through the surgical procedures, with spinal and operative trauma furthering the adverse effects. Assisted or controlled respiration by manual methods failed to compensate for these severe changes in compliance, as monitored by the Liston Becker infrared carbon dioxide analyzer.

With turning of the patient without thoracic deformity to the supine position following surgery, a rapid return to presurgical values occurred. Similarly, the patient with thoracic deformity, although showing some increase in compliance when turned to the supine position, required assisted respiration for much longer periods before returning to the preoperative control value. It is suggested that a controlled volume type respirator be used in these patients, because it automatically adjusts its pressure so that selected tidal volume will reach the patient despite moment-to-moment changes in compliance. Such a respirator should be capable of delivering pressures in excess of 40 mm. of mercury.

E. Shabart

PUBLIC HEALTH AND EPIDEMIOLOGY

Uncommon Skin Reactions After BCG Vaccination. W. Hartston*. *Tubercle*, August, 1959, 40: 265–270. (*Public Health Dept., London County Council, England)

In addition to describing the "normal" local reaction of BCG vaccine, severe acute and chronic inflammatory reactions as well as general skin rashes and BCG scar anomalies are described. Of particular interest are a "sarcoid" pattern of reaction, granulomas, lupus infiltrations, and one case of a histiocytoma diagnosed among 70,000 vaccinated London school children. General rashes include urticaria, erythema nodosum, and an eruption resembling lupus miliaris disseminatus faciei.

M. J. Small

BCG Vaccination and the Depot Tuberculin Test. G. M. Short*. *Tubercle*, August, 1959, 40: 271–274. (*Uganda Med. Dept., Entebbe, Uganda, East Africa)

Twenty-four tuberculin-negative reactors in a group of students at Makerere College, Uganda, were vaccinated with Glaxo's freeze-dried BCG vaccine. PPD tuberculin was diluted and added to a liquid paraffin lanolin vehicle to prepare depot tuberculin, of which the skin test dose contained 5 tuberculin units (TU). The vehicle itself was used for control. Nineteen of the students were given intradermal injections of depot tuberculin (5 TU) and depot material as a control.

Eighteen showed positive reactions at the depot test site between 13 and 22 days after vaccination; none then showed reactions at the control site. One student did not report. Fifty-six days after testing, all 18 showed reactions at both sites; in all cases the tuberculin reaction was greater than the control. A similar picture was seen in all 19 students after 6 months, except for 1 case in which the reactions were equal. At one year, all were still positive at the test site, and most still had reac-

tions at the control site. Some reactions had increased in size at both sites. Increase in size of the depot tuberculin and control reactions was observed in 1 case following re-test by the Heaf method. The persistent reactions caused no physical or mental distress.

The advantages of using the depot tuberculin method as an indicator are obvious. The test can be used to distinguish tuberculin-sensitive and -insensitive individuals by the initial reaction; the tuberculin insensitive can then be vaccinated and told to report if no reaction has appeared at the test site within a specified number of weeks. The time spent in testing is less, and the number of visits reduced. The lower limit of positivity of this test is less than 6 mm.; it appears that a reaction of any size at the depot site may be considered positive.

It is concluded that the depot tuberculin test is an effective and acceptable indicator, in the African, of tuberculin conversion. It is also concluded that the rapidity and thoroughness of tuberculin conversion with Glaxo's freeze-dried BCG vaccine are probably as good as that obtained with fresh liquid vaccine.

M. J. SMALL

Active Tuberculosis in BCG-Inoculated Children (in German). N. EBERS*. *Tuberkulosearzt*, June, 1959, 13: 397–399. (*Elisabethenberg, Waldhausen bei Schorndorf/Württ., Germany)

From 1948–1958, 56 BCG-inoculated children were admitted to a sanatorium. Examination revealed an active process in only 13 cases. Only 5 of these were proved to be failures of BCG vaccination. The other 8 cases probably were exposed to tuberculous infection before the BCG immunization had developed to maximal protective power. Only 1 case was serious; the other 4 were benign primary tuberculosis cases. This number was an exceedingly small fraction of the total number of tuberculous children (800) who were admitted to the sanatorium during the same period.

J. HAAPANEN

Long-term Effectiveness of B.C.G. Vaccination of Infants in Close Contact with Infectious Tuberculosis. J. LORBER* and P. C. MENNER. *Brit. M. J.*, June 6, 1959, No. 5135: 1430–1433. (*U. of Sheffield, England)

Two hundred and sixty-seven children aged 2 years or less who were in close household contact with tuberculous persons, and who remained tuberculin negative 6 weeks after segregation from contact, were vaccinated with BCG between October, 1949, and October, 1952. Of these, 261 were traced 5 years or more after vaccination. There were no deaths from tuberculosis; only 2 had minor clinical symptoms associated with tuberculous lung lesions which healed by calcification, and 1 other had a calcified lung lesion. There were only 4 who reverted and became tuberculin negative. In contrast, of the "index" cases, 39 (15.7 per cent) died of tuberculosis, and 28 (11.3 per cent) still had active disease at the time of this review. As there were no comparable controls, it cannot be shown conclusively that BCG vaccination prevented tuberculosis in this group of infants. Nevertheless, the results are highly suggestive that this, in fact, was the case (after authors' summary).

E. A. RILEY

BCG Vaccination in Sweden. G. DAHLSTROM*. *Tubercle*, June, 1959, 40: 196–200. (*Soderby Sjukhus, Stockholm, Sweden)

The value of BCG vaccination as a means of combating tuberculosis must be judged with strict attention to variations in the prevalence of the disease. Even with the low prevalence which now exists in the Scandinavian countries, it would seem correct to continue with general vaccination of adolescents who are about to leave school and take up employment and, also, of tuberculin-negative defense personnel.

Vaccination of familial contacts of tuberculous persons, hospital staff, et cetera, by tuberculosis dispensaries should be retained indefinitely. The same applies to the armed forces, in which the frequency of tuberculosis has always been higher than among the civilian population. Voluntary vaccination of conscripts is well accepted and this, together with intensive mass roentgenography, has contributed to substantial reduction of the tuberculosis morbidity rate in the forces. On the other hand, there is reason to hope that the tuberculosis situation in Sweden will improve so much within the next few years that the advisability of BCG vaccination of infants may be reconsidered. It would seem that, in the near future, we shall be able to control tuberculosis to such an extent that only a few infants or young children are exposed to risk of infection and therefore require BCG vaccination.

M. J. SMALL

Current Problems of Tuberculin Reaction. (Should the Current Criterion of Positive Reaction be Changed?) (in Japanese). H. OKADA* and M. ASANO. *Jap. J. Clin. Tuberc.*, August, 1959, 18: 555–564. (*Nagoya U., Nagoya, Japan)

Widespread and repeated application of tuberculin tests and BCG vaccination in tuberculosis-prevention campaigns, which changed the allergic reaction to tuberculin of many individuals, has

apparently outdated the old criterion of positive tuberculin reaction which was established in the 1930's when few people were either tested or vaccinated. This is reflected in the frequent observations of weak and ambiguous reactions, accelerated reactions at the site of "repeatedly tuberculin tested" portions of the arm, and delayed positive reactions.

The old criterion of positive reaction, i.e., more than 10 mm. of redness at 48 hours, may still hold true in those who are tuberculin tested for the first time, but not in those who were BCG vaccinated and are frequently tuberculin tested. Weak and ambiguous reactions are more often observed at the frequently tuberculin tested site than at the less frequently tested site, irrespective of whether the individual has been BCG vaccinated, and the reaction is usually maximal at 24 hours at the former and at 48 hours at the latter site, respectively. Delayed positive reaction is considered to be a specific allergic reaction.

I. TATENO

Tuberculin Sensitivity and Tuberculosis Among Natives of the Lower Yukon. G. W. COMSTOCK* and M. E. PORTER. *Pub. Health Rep.*, July, 1959, 74: 621–634. (*Arctic Health Research Center, Anchorage, Alaska)

In 1957, tuberculin tests were done on 2,211 natives living in 19 villages along or near the Yukon River in Alaska. The prevalence of positive tuberculin reactions increased markedly with age, reaching a level of 96 per cent in the age group 15 to 19 years. Among unvaccinated adult reactors to the 5-TU dose, the prevalence of active and inactive reinfection-type tuberculosis was almost 30 per cent, and was essentially the same among persons with small and large tuberculin reactions. In contrast, in Muscogee County, Georgia and Russell County, Alabama, where there is a great deal of nonspecific sensitivity, the prevalence among unvaccinated adult reactors was 1.2 per cent, and was much higher among persons with large reactions than among those with small reactions. This finding is interpreted to signify that the prevalence of tuberculosis does not vary with the degree of allergy from specific infection, and is consistent with the concept that there is considerable nonspecific tuberculin sensitivity in the Muscogee-Russell area and little if any in the lower Yukon area. The problem of defining a positive tuberculin reactor is discussed, and it is suggested that the definition selected should vary according to the prevalence of nonspecific sensitivity in the area under consideration.

E. DUNNER

Cancer of the Lung in Tuberculous Patients. N. C. HAMEL*, J. N. BRIGGS, and L. G. LUDINGTON. *Calif. Med.*, September, 1959, 91: 131–133. (*Encino, California)

Of all tuberculous patients over 45 years of age admitted to Olive View Sanatorium in the 5-year period ended July, 1958, 1.4 per cent had cancer of the lung. This is a much higher incidence than in a comparable segment of the general population: it is estimated to be 8 to 10 times as great. The most important diagnostic clues were obtained from careful studies of serial roentgenograms. Scalene node biopsy, bronchoscopy, and cytologic studies of sputum yielded disappointingly little information. Twenty-five of the 54 cases were considered operable as compared with 21 of 85 cases in nontuberculous patients observed at the same institution.

E. A. ROUFF

Health Hazards of Automobile Exhaust. J. R. GOLDSMITH* and L. H. ROGERS. *Pub. Health Rep.*, June, 1959, 74: 551–558. (*California State Dept. of Public Health, Berkeley)

Of the substances which occur in automobile exhaust and their reaction products, hygienic standards have been established for industrial exposure to carbon monoxide, nitrogen dioxide, lead, and ozone. Establishing a full set of levels for community exposures to these substances is very difficult because of the sensitivity of frail or ill individuals, the indeterminate period of exposure, the effect of agents in combination, and the cumulative effect of exposure from other sources, such as cigarette smoking. In the absence of effective control for air pollution from automobile exhaust, the public health hazard should be evaluated.

E. DUNNER

Tobacco Consumption and Mortality from Cancer and Other Diseases. H. F. DORN*. *Pub. Health Rep.*, July, 1959, 74: 581–593. (*National Institutes of Health, Bethesda, Md.)

This report summarizes the mortality experience of nearly 200,000 policyholders of U. S. Government life insurance from July, 1954 through December, 1956. All of these policyholders served in the Armed Forces of the United States between 1917 and 1940.

The death rate from all causes for men who have used tobacco is 32 per cent greater than that for men who have never smoked. Men who have regularly smoked only cigarettes have the highest death rate of all groups of smokers, 58 per cent greater than the rate for nonsmokers. The death rate from all causes for men who have regularly smoked cigars or a pipe, or both, is not appreciably higher than that for nonsmokers. Regular cig-

arette smokers who had stopped smoking cigarettes before the study began in 1954 have a lower mortality rate than those who continued to smoke. However, the rate for the ex-smokers still is 31 per cent greater than that of nonsmokers. The excess mortality of regular cigarette smokers is greater for heavy smokers than for light smokers. Only the heaviest users of cigars and pipe tobacco experience a significant increase in total mortality over that of men who have never smoked. The greatest increase for smokers in the risk of developing a disease is for cancer of the lung. The mortality ratio for regular smokers of cigarettes only is 9.85, or about 10 times that for nonsmokers. Regular users of cigars or pipes, or both, have an increased mortality rate for cancer of the lung as well as for all forms of cancer as a group, but this increase is much less than that for cigarette smokers. Regular cigarette smokers are subject also to an increased risk of dying from cardiovascular disease, from certain respiratory diseases such as bronchitis, pleurisy, and emphysema, from ulcers of the stomach and duodenum, and from cirrhosis of the liver. The death rate from coronary heart disease among regular users of cigarettes only is 63 per cent higher than the rate for nonsmokers.

The leading cause of death among the policyholders is coronary heart disease. Forty-four per cent of the number of observed deaths and 45 per cent of the number of excess deaths—the difference between the number of observed deaths and the number of deaths expected on the basis of the rates for nonsmokers—for smokers of cigarettes only, were attributed to coronary heart disease. Corresponding percentages for lung cancer were 7 and 17.

E. DUNNER

Influenza. *Bull. World Health Organ.*, Nos. 2-3, 1959, 20: 183–508.

This issue brings together a number of the reports sent to the World Health Organization from various European countries, as well as India, Japan, and French Equatorial Africa, regarding the influenza epidemic of 1957. In a series of 19 articles, epidemiologic aspects, vaccination, diagnostic tools, and related problems are discussed.

H. ABELES

Observations on Asian Influenza on Two Alaskan Islands. R. N. PHILIP*, W. T. WEEKS, K. R. REINHARD, D. B. LACKMAN, and C. FRENCH. *Pub. Health Rep.*, August, 1959, 74: 737–745. (*Aretic Health Research Center, Public Health Service, Anchorage, Alaska)

Asian influenza outbreaks occurred during October and November, 1957, in the remote communities of St. Paul, Pribilof Islands; and Gambell, St. Lawrence Island, Alaska. The introduction of the virus into each village could be dated precisely and in each instance was attributed to a returning serviceman. Two hundred and ninety-four (82 per cent) of 358 St. Paul residents and 262 (87 per cent) of 300 Gambell Eskimos were ill during the outbreaks. Attack rates were higher among the Aleut than the white residents of St. Paul. Attack rates were higher among children than adults, but the difference was not so great as generally noted with Asian influenza. The disease was generally mild and clinically similar to influenza noted elsewhere. There were 2 deaths in St. Paul. Pulmonary complications were somewhat more frequent than generally noted. There was, however, no evidence that such complications were viral in origin or that the epidemic strain of virus was more virulent than observed elsewhere.

E. DUNNER

Isolation of a Haemadsorption Virus from the Recent Outbreak of Respiratory Illness in Ontario. N. A. LABZOFFSKY*, M. R. COOPER, L. P. MORRISSEY, and J. LESIAK. *Canad. M.A.J.*, July 15, 1959, 81: 110–112. (*Virus Diagnostic Unit, Central Lab., Ontario Dept. of Health, Canada)

During the recent outbreak of influenza-like illness in Ontario, a hemadsorption virus was isolated. This virus was found to be different from any of the influenza viruses, Newcastle disease virus (NDV), mumps, Copenhagen 222, and hemadsorption Type II viruses. Further, it has been established that it is serologically related but not identical with hemadsorption Type I virus (Authors' summary)

E. A. RILEY

Frevention of the Development of Pulmonary Tuberculosis by Oral Administration of Isoniazid in Children (in Japanese). S. TAZAKI*. *Clin. Resp. Organs*, August, 1959, 14: 611–618. (*Dept. of Pediatrics, Nihon U. School of Med., Tokyo, Japan)

Of 47,402 patients who were tuberculin tested every 3 months, 248 were detected whose tuberculin reactions became positive following natural infection. They were arbitrarily divided into two groups to study the preventive effect of isoniazid in the development of pulmonary tuberculosis, and were observed for 3 to 4 years.

Among 119 patients who received 4 mg. per kg. of isoniazid for 90 days, none developed tuberculosis, while 4 of 120 control patients developed illness. The intensity of the tuberculin reaction, sedimentation rate, or body weight was comparable for the 2 groups except in those who actually developed pulmonary tuberculosis. Isoniazid was

considered an effective preventive drug in natural tuberculin-positive converters.

I. TATENO

Administration of Isoniazid for the Prevention of Pulmonary Tuberculosis in High School Students (in Japanese). Y. KASAI*, M. KUCHIKI, and S. KUBOTA. *Clin. Resp. Organs*, August, 1959, 14: 619–628. (*Sapporo Kenko Sodanjo, Sapporo, Japan)

Senior high school students who were busy with school work and also liable to the development of pulmonary tuberculosis were selected for the study of the protective effect of isoniazid compounds. The conclusions reached were: (*1*) Isoniazid compounds were effective in the prevention of the disease. (*2*) In view of the fact that some cases from treated groups developed tuberculosis, however, on a daily dose of 0.3 gm. of Na methansulfonate of isoniazid given twice a week, it was considered necessary to change the minimal treatment schedule to a larger dosage or administer medication every other day. Isoniazid was cheaper than Na methansulfonate of isoniazid, but the side effects may be less with the latter and, therefore, it is safer for prophylactic purposes.

I. TATENO

Studies on the Effect of Isoniazid upon the Antituberculous Immunity Induced by BCG Vaccination. M. TOYOHARA*, S. KUDOH, and Y. OBAYASHI. *Tubercle*, June, 1959, 40: 184–191. (*Research institute, Japan Anti-Tuberculosis Association, Tokyo)

Results are presented of a study on the effect of isoniazid administration on antituberculous immunity. The administration of isoniazid before BCG vaccination considerably reduced both the development of immunity and of tuberculin sensitivity. There was no significant difference in the degree of immunity between this group and the group vaccinated with heat-killed BCG, although tuberculin sensitivity was higher with the former than with the latter. When isoniazid was given 2 weeks after BCG vaccination, the development of antituberculous immunity seemed unaffected by this procedure, although the development of tuberculin sensitivity was definitely reduced. The degree of immunity in the group infected 2 weeks after the vaccination was obviously lower than that of the group which was infected after a further 4 weeks' contact of BCG organisms with the host under the administration of isoniazid. Tuberculous changes were less in the group vaccinated with heat-killed BCG than in the nonvaccinated control group. It is concluded that the increase of bacterial organisms due to the multiplication in the host plays an important role in the acquisition of antituberculous immunity.

M. J. SMALL

Lepromin Sensitivity in Different Forms of Pulmonary Tuberculosis (in German). F. KOVÁTS* and R. BÖSZ. *Tuberkulosearzt*, June, 1959, 13: 385–387. (*U. of Budapest, Hungary)

A positive lepromin skin reaction is considered indicative of immunity against tuberculosis in tuberculous patients. The test was performed on 75 patients. In cases of localized, chronic pulmonary tuberculosis the lepromin and tuberculin reactions were equal. In hematogenous forms the lepromin reaction was positive in only 50 per cent, against 100 per cent positivity for tuberculin. In 6 cases of pleurisy, the lepromin reactions were negative while the tuberculin reactions were positive. In 9 cases of sarcoidosis and 2 cases of Hodgkin's disease, both reactions were negative.

J. HAAPANEN

Data on the Epidemiology of Bovine Tuberculosis (in Hungarian). GY. BERENCSI* and N. RADNAI. *Tuberkulózis*, October–December, 1958, 10–12: 272–273. (*U. of Debrecen, Hungary)

In the city of Püspökladány, the cattle were tuberculin tested. Positive tuberculin reactions were found in 177 animals (13.7 per cent), of which 9 had active open tuberculosis (5.1 per cent). These figures roughly correspond to the percentage of tuberculosis in the population of the country.

Z. VIRÁGH

Intradermal Vaccination with the Vole Bacillus. A. Q. WELLS* and J. A. H. WYLIE. *Tubercle*, June, 1959, 40: 181–183. (*Brompton Hosp., London, England)

Vole bacillus vaccination by the intradermal route is described. Two accurately measured strengths of vaccine were used, and the superiority of a near unicellular suspension demonstrated. The conversion rates and, particularly, the persistence of a positive Mantoux reaction compare favorably with BCG. The dramatic advances in treating tuberculosis in recent years and the still equivocal value of protective vaccination in particular circumstances, irrespective of the vaccine or the technique of administration used, have combined to lessen the importance of this form of prophylaxis in civilized communities. The technique reported here, however, has certain advantages in that remarkably accurate dosage can be achieved and the incidence of complications with the smaller dose is much less than when the multiple-puncture technique was employed.

M. J. SMALL

Tuberculosis Contacts in North-West London. P. A. Emerson*. *Tubercle*, June, 1959, 40: 177–180. (*Westminster Hosp., London, S.W. 1, England)

The present study was undertaken to assess the incidence of tuberculosis among the household contacts of infectious tuberculous patients, first, at the time these index cases ceased to be infectious and, second, in the following 5 years. Based on a study of 1,517 household contacts of 555 patients with positive sputum, it is recommended that contacts of all ages who are found or assumed to be tuberculin positive should have a chest roentgenogram taken initially, and this examination should be repeated at least once, a year later.

Because of the serious consequences of tuberculosis in children under 5, the repeat examination should be done every 3 to 4 months for the first year.

Some 5.6 per cent of all of these contacts of infectious patients may be expected to have tuberculosis at their initial examinations and, of those with initially normal chest roentgenograms, another 1.9 per cent may be expected to develop tuberculosis in the following year. Thereafter, yearly chest roentgenograms of those contacts below the age of 35 years may still be expected to give yield well in excess of the annual attack rate (0.2 per cent) of the average urban population. Yearly chest films should, therefore, be advised. Children in the 6–15-year age group require the same attention as the older age groups.

M. J. Small

Present Status of Childhood Tuberculosis in Czechoslovakia (in Hungarian). V. Vojtek*. *Tuberkulózis*, October–December, 1958, 10–12: 244–248. (*TB Sanat. Sumperk, Czechoslovakia)

In Czechoslovakia, the fight against tuberculosis is the responsibility of the Ministry of Health. Directed and supervised centrally, each district (there are 22 districts in the country, each with about 600,000 people) has a phthisiologist who is assisted in the problems of childhood tuberculosis by a phthisio-pediatrician. There are 2,100 sanatorium beds for tuberculous children, 591 of them for bone and joint cases. BCG vaccination has been compulsory since 1943. Revaccination is done at 6, 10, 14, 19, 25, and 30 years of age. The number of tuberculous children in 1954 was 4,434 while in 1957 it was 2,803. Similarly, the number of children with tuberculous meningitis decreased from 284 to 190 in the years mentioned. All forms of collapse therapy and thoracic surgery are performed; among the antimicrobials, isoniazid, PAS, and streptomycin are employed.

Z. Virágh

Tuberculosis Control in Denmark. H. Franks*. *Brit. M. J.*, July 25, 1959, No. 5142: 88–92. (*Medical Officer of Health, Chigwell and Waltham Holy Cross Urban District Councils, England)

Methods of tuberculosis control and the incidence of tuberculosis are compared in Denmark with those in England and Wales. The incidence of tuberculosis has fallen faster and further in Denmark than in England and Wales. This appears to be due to a policy based on the belief that the disease can be entirely eradicated in the reasonably near future, and differs from the British procedures in the following main respects: (*1*) the maintenance of a Central Tuberculosis Register; (*2*) extensive examination of sputum and stomach washings in a specialized central laboratory; (*3*) extensive use of BCG at all ages and in conjunction with mass roentgenography; (*4*) selective use of mass roentgenography, including annual examination of all staff employees in schools, public utilities, et cetera, as part of their terms of contract; (*5*) invitations to public mass roentgenography sessions to individual households by post; (*6*) wider use of powers for compulsory isolation; (*7*) unified control (after author's summary).

E. A. Riley

The Problem of Tuberculosis in Turkey. N. Karasu*. *Tubercle*, August, 1959, 40: 275–287. (*Ankara U., Turkey)

Turkey is attempting to find the best, cheapest, and fastest method to fight tuberculosis. Because there are only 11,000 hospital beds for 250,000 active cases, general opinion is not in favor of extended hospital isolation. A large-scale case-finding program, together with home care, chemoprophylaxis, and BCG vaccination, will be the basic principles of the campaign. It is not easy to carry on this program, for large proportions of the population live in villages under very primitive sanitary conditions.

The very good results in Ankara City after the establishment of regional dispensaries which have close cooperation with the well-developed central laboratories show that the best way will be by opening regional dispensaries throughout Turkey, 1 for every 50,000 people, as soon as possible. To start a country-wide, effective tuberculosis campaign, the help of WHO and UNICEF is needed. Working together in the fight against one of the worst enemies of mankind will surely be one of the best signs for the future of humanity.

M. J. Small

WHO and Tuberculosis. Leading Article*. *Tubercle*, June, 1959, 40: 222–223. (*Official Records of the World Health Organization, No. 90, WHO, Geneva, Switzerland)

The year 1958 marked the tenth anniversary of the World Health Organization. The Annual Report of the Director-General lists many projects concerned with tuberculosis. The mere list of countries indicates the wide scope of the program. In the African Region, prevalence surveys, mass BCG vaccinations, or other control measures are being assisted in Tanganyika, Uganda, Kenya, Nigeria, Sierra Leone, Gambia, and Basutoland. In Mauritius, a tuberculin testing, roentgenographic, and sputum examination survey of a small random sample was preceded by a house-to-house population census. In Bechuanaland, the BCG vaccination program was to start in August, for that is the time when the semi-nomadic population is temporarily settled. A sample survey of 15 per cent of the population of St. Helena showed the lowest prevalence of tuberculosis of any country so far surveyed by WHO teams. The list continues with Guatemala and Honduras in the American Region; and, in the East Mediterranean Region, Jordan, Lebanon, Sudan, and Iran, it is reported that 44 vaccination teams carried out 87,000 tests a month. In Pakistan, 9 million have already been vaccinated and vaccination of a further 4½ million is planned in the next 2 years. The Western Pacific Region contains only two projects, in Viet Nam and Formosa; but in the South East Asia Region, there are projects in Afghanistan, Burma, Ceylon, Indonesia, Thailand, and India. In India, assistance is being given with the Tuberculosis Control and Training Centers at Nagpur and Hyderabad, and plans have been accepted by the Government for a National Tuberculosis Program, providing for state district tuberculosis centers and a national tuberculosis center for coordination, training, and field studies. In India, too, there is the important Tuberculosis Chemotherapy Center—a joint enterprise of the Indian Council of Medical Research, WHO, and the British Medical Research Council. Here, in Madras, there are in progress, among the other investigations, comparative studies of the results of treatment in hospital and at home, comparisons of different treatments given in the home, socio-economic studies on a group of patients, and investigations of the incidence of tuberculosis in family contacts. This is only a partial listing.

Of interest is the report on the control program in Iran where it is stated that the Anti-tuberculosis Demonstration Center in Teheran has, after many difficulties, been established; but "no immediate or spectacular improvement of the tuberculosis situation should be expected, since the needs are greater than the present resources. . . ."

M. J. Small

ROENTGENOGRAPHY

Percutaneous Selective Angiography of the Internal Mammary Artery. O. Arner*, P. Edholm, and P. Odman. *Acta radiol.*, June, 1959, 51: 433–438. (*Karolinska Sjukhuset, Stockholm, Sweden)

A technique, under fluoroscopic control, of percutaneous catheterization of the internal mammary artery using a KIFA model polythene catheter introduced into the brachial artery is discussed in detail. Fifteen cubic centimeters of Urografin (45 per cent) is used as the contrast media. Some experiences with the procedure are given. The main object of the investigation was to determine whether any reliable angiographic differences exist in certain tumors of the breast. The results obtained in six cases were indefinite; but the end result proved that a method is made possible to study areas communicating with the internal mammary artery.

E. Shabart

Intralobar Bronchopulmonary Sequestration in Children—Diagnosis by Intrathoracic Aortography. A. P. Simopoulos*, D. J. Rosenblum, H. Mazumdar, and B. Kiely. *A.M.A. J. Dis. Child.*, June, 1959, 97: 796–804. (*Kings County Hosp., Brooklyn, N.Y.)

Intralobar bronchopulmonary sequestration is a congenital anomaly of the lung causing chronic and recurrent infection and requiring surgical removal. It can be identified by the history and appearance on the plain roentgenograms and bronchogram, or by demonstration of the abnormal systemic artery which supplies it by intrathoracic aortography. The location of the involved areas in the posterior basal segment of the lower lobe and the recurrence of disease always in the same part of the lung are suggestive. In 2 cases described in detail it was found that the roentgenographic abnormality had been present since birth, and in both cases changes from a solid to a cystic appearance and vice-versa were observed. These 2 features are typical of intralobar bronchopulmonary sequestration. This diagnosis should always be considered in cases of localized chronic or recurrent pulmonary disease.

M. J. Small

Radiation Hazards to Patients. Leading Article. *Tubercle*, August, 1959, 40: 288–289.

The Ministry of Health recently published an Interim Report by the Adrian Committee, which is chiefly concerned with mass roentgenography.

Their general conclusion is that "the somatic risk of doses at these levels is, on any interpretation, far outweighed by the benefits of mass miniature radiography."

A separate survey was undertaken to estimate skin dose, and from this the main marrow dose has been estimated. Then, assuming that mass roentgenography continues indefinitely, the authors conclude that, on the basis of the skin dose, there will be at most 20 cases of leukemia a year in addition to the present 2,500. These risks are small but they probably exist, and the authors claim that the benefits of mass filming outweigh the dangers to the population. They state that mass roentgenography diagnoses 17,835 cases of pulmonary tuberculosis requiring supervision very year, as well as 2,362 cases of lung cancer, 12,000 cardiac abnormalities, and 9,400 cases of pneumoconiosis. One important factor in eradicating tuberculosis rapidly from England is further use of mass roentgenography, and it might have been wiser if the authors had rested their case on this. It is undoubtedly a very good one.

On the practical side, the Committee makes many sensible points about fluoroscopy, the filming of children and pregnant women, the use of fast films, minimum timing, the use of the smallest reasonable field size, and gonad shields, all of which are exceedingly important.

M. J. SMALL

Observer Disagreements in the Interpretation of Hilar Shadows in Chest Radiographs. A. R. SOMNER*, *Tubercle*, August, 1959, 40: 245–250. (*Northern General Hosp., Edinburgh, Scotland)

As part of an investigation of patients with hemoptysis and "normal" chest roentgenograms, 18 observers accustomed to the interpretation of chest films reviewed the films of 69 patients examined by bronchoscopy to confirm or exclude bronchogenic carcinoma. They were asked to classify the 138 hilar shadows as either "normal," "suspicious," or "definitely enlarged." The clinicians in charge of these patients had previously come to an agreement about the classification of these same hilar shadows. In no case was there any abnormal shadow in the lung field.

The majority of the observers were in agreement about the appearance of the majority of the hilar shadows as defined by the clinicians. Each observer's disagreements with the clinicians, whether many or few, commonly received support from only a minority of the other observers. There were wide differences between the observers in their assessment of the hilar shadows; this emphasizes the difficulty in assessing a normal hilar shadow in a chest film in routine clinical work.

One practical conclusion from this inquiry is that, in studies of observer variability, it should be recognized that the roentgenographic opinion of any 2 or 3 observers may be biased and that the numerical advantage of duplicate or triplicate film readings may be influenced by this factor of personal caution. It would appear that there is less likelihood of the bias occurring if a greater number of observers are included (e.g., the 18 of this survey), because the more extreme degrees of caution or suspiciousness may be balanced by the lower level of suspiciousness of other observers as to what constitutes a "normal" hilar shadow.

M. J. SMALL

Clinical and Roentgenological Signs of Collagen Diseases Involving the Thorax. C. M. NICE, JR.*, A. N. K. MENON, and L. G. RIGLER. *Dis. Chest*, June, 1959, 35: 634–650. (*U. of Minnesota Med. School, Minneapolis)

Clinical and roentgenographic manifestations of involvement of thoracic structures by collagen diseases are more common than has been stressed, including such relatively uncommon conditions as dermatomyositis. Roentgen abnormalities in the chest may quite often lead one to suspect the presence of a collagen disease, and certain combinations of sequential changes may even aid in the differential diagnosis of the various conditions. The following conditions are presented in this context: (*1*) polyarteritis nodosa, (*2*) disseminated lupus erythematosus, (*3*) scleroderma, (*4*) dermatomyositis, (*5*) rheumatic pneumonitis and, (*6*) overlapping or transitional ferus.

E. A. ROUFF

An Experiment in Mass Radiography. J. B. COCHRANE*, W. B. FLETCHER, and C. CLAYSON. *Brit. M. J.*, July 4, 1959, No. 5139: 1–5. (*Lochmaben Chest Hosp., Dumfriesshire, Scotland)

A controlled experiment in mass roentgenography has been carried out in a Scottish burgh (population 26,000). By dividing the town into three sectors and applying different techniques, the influence of home-visiting and census-taking on population response has been measured. The over-all response was 69.7 per cent of the population aged 15 and older. Home visiting increased the response by 20 per cent up to the middle-age groups, and thereafter by about 30 to 40 per cent. There was evidence that the personal contact of a census also increased the response of the elderly. Forty per cent of the males and more than 50 per cent of the females had not been previously roentgenographed. Reasons for nonattendance were analyzed in one sector. Four per cent were found to be physically unfit to come to the roentgenography unit; 15 per cent had legitimate health

reasons for nonattendance, although they were not found physically unfit to attend; 15 per cent had been recently filmed, but not within the last 6 months; and the remaining 60 per cent had no obvious reason for refusing. Twenty-one cases of active tuberculosis (1.5 per thousand filmed) were discovered.

E. A. Riley

Bronchial Carcinoma in Men Detected by Selective and Unselective Miniature Radiography: A Review of 228 Cases. G. Z. Brett*. *Tubercle*, June, 1959, 40: 192–195. (*North West Metropolitan Regional Hosp. Board, London, N.5, England)

An analysis of 228 cases of bronchial carcinoma in men, discovered by selective and unselective roentgenography during 1952–1956, is reported. The high rate of detection in those presenting chest symptoms at a static miniature roentgenography unit (selective roentgenography) was compared with the low yield in assumed healthy examinees attending a mobile unit (unselective roentgenography). There is a strong suggestion that the patients discovered by the unselective method have a greater chance of having the cancer removed and of surviving than the patients with symptoms who are discovered by the selective method. These figures support the view of those who believe that the search for the disease should be concentrated on "silent" cases, for which purpose unselective roentgenography, properly adapted, could be the method of choice.

M. J. Small

CHEMOTHERAPY

Relapse of Pulmonary Tuberculosis after Treatment with Antimicrobials and Intrapleural Pneumothorax (in Italian). M. Lucchesi*, M. Zubiani, and L. Catacchio. *Lotta contro tuberc.*, May, 1959, 29: 589–618. (*Istituto C. Forlanini, Rome, Italy)

Of 7,980 patients with pulmonary tuberculosis who were treated with antimicrobials alone, or in combination with pneumothorax, 892 apparently recovered patients were selected. Of these, 647 were followed and it was found that 70 (10.81 per cent) had had relapses.

The frequency of relapse was higher in males (13.79 per cent) than in females (8.43 per cent), in persons older than 40 years, in patients with diffused exudative processes—higher in those with bilateral lesions (17.11 per cent) than unilateral (5.44 per cent). The frequency of relapse was also strictly bound to the antimicrobial therapy: the patients treated with high-dosage streptomycin and isoniazid had a low incidence of relapse. The relapses were more frequent in the first two years of recovery; therefore, it is recommended that a few cycles of therapy be instituted, particularly with isoniazid. The prevalent anatomic position of relapses was the same as that of the first attack (86.30 per cent); the more frequent clinical form was that with cavitary lesions (62.32 per cent). Bacteriologic positivity was very high (82.65 per cent), with the frequent finding of resistant bacilli.

I. Archetti

Studies on the Combined Use of Antituberculous Drugs and Corticosteroids in the Treatment of Pulmonary Tuberculosis, Tuberculous Pleurisy, and Meningitis (in Japanese). Committee on the Study of Corticosteroids in Infections under the Auspices of the Ministry of Education, represented by O. Kitamoto*. *Saishin Igaku*, August, 1959, 14: 2237–2248. (*Institute for Infectious Diseases, Tokyo U., Japan)

The effect of the combined use of corticosteroids and antituberculosis drugs in pulmonary tuberculosis (131 cases), tuberculous pleurisy (71 cases), and meningitis (11 cases) were studied for 2 months, 2 months, and 1 to 4 months, respectively. The effect was analyzed from every aspect of the clinical, roentgenographic, and bacteriologic viewpoints.

This type of treatment was especially effective in the A (exudative), B (infiltrative-caseous), and E (disseminated) types of pulmonary tuberculosis. Tubercle bacilli disappeared more rapidly, and decrease of body temperature and increase in appetite were more remarkable with this treatment than with antimicrobials alone. Corticosteroids did not have a beneficial effect on other types of pulmonary tuberculosis. Rapid defervescence and absorption of exudates were observed in exudative pleurisy both by oral or intrathoracic administration of steroids. Defervescence, improvement of meningeal symptoms, and recovery of mental disturbances were rapid in tuberculous meningitis with this treatment.

I. Tateno

Swiss Phthisiologists' Report on Clinical Trial of Cycloserine (in German). N. Pult* and S. Barandun. *Schweiz. Ztschr. Tuberk.*, No. 6, 1958, 15: 417–468. (*Clinique Militaire Fédéral, Montana, Switzerland)

A team of 24 Swiss physicians representing numerous tuberculosis hospitals conducted a clinical experiment with cycloserine for 6 months in 2 groups of patients. The first group consisted of 82 patients of both sexes aged 14–78 years. They were moderately or far advanced chronic cases

treated unsuccessfully with various antimicrobials, collapse therapy, and surgery. Most were resistant to isoniazid (Rimifon®) and streptomycin, and partially resistant to PAS. These patients received cycloserine alone in daily doses of 750 mg. There were 43 patients in the other group, also with moderately or far advanced tuberculosis, positive for acid-fast bacilli, not treated with antimicrobials previously. They were given 750 mg. of cycloserine and 300 mg. of isoniazid daily.

In the first group, roentgenographic improvement was recorded in 14 per cent, and secretions became negative for acid-fast bacilli in 38 per cent of the patients. The respective figures in the isoniazid-cycloserine group were 61 and 74 per cent. Improvement of patients on cycloserine alone was noted mostly in the first 3 months, afterward the course became stationary; in some cases regression was observed. In the second group, the improvement was continuous, regression was very infrequent. Specific and toxic reactions occurred in both groups. More than half of the patients treated with cycloserine alone developed resistance to the drug in the second month of treatment; the number increased with time.

Treatment with cycloserine is contraindicated in exudative tuberculosis with fever and immediately before or after surgical intervention. Because of specific reactions, caution is recommended in cases of kidney insufficiency; similarly in patients with epilepsy, psychoneuronosis, alchoholism, and those in generally weak conditions.

Z. Virágh

Dihydrostreptomycin Deafness. G. E. Shambaugh, Jr.*, E. L. Derlacki, W. H. Harrison, H. House, W. House, V. Hildyard, H. Schuknecht, and J. J. Shea. *J. A. M. A.*, August 1, 1959, 170: 1657–1660. (*Chicago, Ill.)

Permanent hearing loss after the use of dihydrostreptomycin was observed in 32 patients. There was a latent period of from several weeks to as long as 6 months between the administration of the drug and the onset of the hearing loss. The hearing loss was in some cases caused by such small doses as 2 or 3 gm. of dihydrostreptomycin. There is no known effective treatment for this type of nerve deafness.

H. Abeles

Activity of Kanamycin on Experimental Tuberculosis in Guinea Pigs (in Italian). A. Blasi*, G. Curci, and V. Guidi. *Arch. tisiol.*, April, 1959, 14: 286–301. (*U. of Naples, Italy)

Four groups of about 10 guinea pigs each were inoculated subcutaneously with 0.10 mg. of *M. tuberculosis*, strain H37Rv. Excepting the animals of the control group, they were treated with kanamycin, given in one daily dose of 20 mg., starting from the day of inoculation or 33 days after, or in two doses of 10 mg. each, for a period of about a month.

The results were based on gross anatomic and histologic examinations. It was found that treatment with 20 mg. given in one dose from the day of infection cured the disease, while when the antimicrobial was divided in two doses the efficacy was less evident. The animals treated with one dose of 20 mg. starting on the 33rd day of disease showed a picture very similar to that of the controls.

I. Archetti

The *in Vivo* and *in Vitro* Activity of Ristocetin upon *M. Tuberculosis* (in Italian). G. Spina*, A. Cassano, G. Storniello, and L. Nuzzolo. *Ann. Ist. Carlo Forlanini*, No. 4, 1958, 18: 409–430. (*Istituto C. Forlanini, Rome, Italy)

The *in vitro* and *in vivo* activity of ristocetin, an antimicrobial isolated from *Nocardia lurida*, was studied against two normal virulent strains of *M. tuberculosis*, 1 bovine strain, 1 paratuberculous strain, and a streptomycin- and an isoniazid-resistant strain. The bacilli were grown in Dubos medium and after different lengths of time of contact with the substance were tested in Petragnani solid medium. The *in vivo* experiments were done in rabbits, which were inoculated intradermally with the bovine strain and treated with the antimicrobial.

The data obtained so far support the concept that ristocetin possesses activity upon Koch's bacilli *in vitro* and, notably, *in vivo*. According to these results, a human trial could start soon.

I. Archetti

Viomycin Pantothenate in the Treatment of Tuberculosis (in Italian). C. Grassi*, F. Lavorato, G. Perna, C. Sacerdoti, and G. Tansini. *Gior. ital. tuberc.*, May–June, 1959, 13: 129–137. (*U. of Milan, Italy)

Fifty-two patients with cavitary tuberculosis of the lung, all with sputum positive for tubercle bacilli, 20 of whom had been previously treated for various periods with other antimicrobials, were given viomycin pantothenate either intramuscularly or by intravenous perfusion in a daily dosage of 2 gm., 3 days a week, for a period of 90 to 150 days. Clinical and roentgenographic improvement, substantiated by laboratory findings, resulted almost unanimously. Tolerance to the antimicrobial was usually good.

I. Archetti

Problems of Long-term and Combination Therapy of Tuberculosis. The Value of Combination Therapy with Thiosemicarbazones (in German). F. JIRA*. *Wien. klin. Wchnschr.*, June 5, 1959, 71: 408–413. (*Strengeberg, Puchberg am Schneeberg, Austria)

Thiosemicarbazone is effective against tubercle bacilli resistant to isoniazid. In addition, it helps maintain a higher isoniazid level in the blood. Combined therapy was given to 636 patients with tuberculosis, 632 with the pulmonary, and 4 with the extrapulmonary form of the disease. Neohydrazon® (tablets containing 0.04 gm. of isoniazid and 0.01 gm. of thiosemicarbazone-benzaldehyde-4-acetylamid), 5 to 8 mg. per kg. daily, was given to 313 of the patients. The same medications, given separately, however, were given to 323 of the patients. The regimens were administered for periods of 1 to more than 11 months. Very good results with very rare adverse side effects were achieved.

G. C. LEINER

The Superiority of Enzyme Impregnated Paper for Determining Glycosuria in Patients Receiving Antituberculosis Drug Therapy. R. W. PHILLIPS*. *Dis. Chest*, August, 1959, 36: 160–163. (*V. A. Hosp., Providence, Rhode Island)

The presence of reducing substances other than glucose in the urine of patients receiving antituberculosis drug therapy is a relatively common occurrence. Of 40 patients on drug therapy, 75 per cent were found to have false positive reactions for glycosuria with the copper reduction Benedict test. None had false positive reactions with the enzyme-impregnated paper test. Blood sugar determinations in each patient were normal.

E. A. ROUFF

PULMONARY PHYSIOLOGY

Dyspnea and Respiratory Work: Studies on the Mechanics of Respiration during Hyperventilation and during Hard Physical Exercise (in German). P. H. ROSSIER* and A. BÜHLMANN. *Schweiz. med. Wchnschr.*, May 23, 1959, 89: 543–548. (*U. of Zürich, Switzerland)

Respiratory work was determined by measurement of intraesophageal pressure and by pneumotachography at different ventilatory volumes, ranging from 5 to 125 liters per minute, in 7 healthy young males during physical exercise on a bicycle ergometer and during voluntary hyperventilation.

In a range from 10 to 100 liters, the respiratory work per liter showed an approximately linear rise which reflected the progressive rise of the flow resistance with increasing flow. Consequently, the respiratory work per minute increased exponentially and called for a commensurate rise of oxygen consumption by the respiratory muscles. Since there is an approximately straight-line relationship between ventilation and oxygen uptake, and because of the exponential rise in expiratory work with increasing ventilatory volumes, a critical point will be reached with large ventilatory volumes in which the oxygen uptake will be consumed mainly by the respiratory muscles; consequently the body will not further profit from any rise in ventilation. For normal individuals this point is reached between 130 and 140 liters per minute. Such ventilatory volumes can be attained and even exceeded only during voluntary hyperventilation. They are usually not the result of physical exercise. There is a fall of alveolar partial pressure of carbon dioxide during voluntary hyperventilation up to a ventilatory volume of 60–70 liters per minute. Beyond these volumes the appreciable rise in carbon dioxide production by the respiratory muscles prevents a further fall in the partial pressure of carbon dioxide.

In certain pathologic conditions, e.g., in obstructuve emphysema, respiratory work is already elevated at rest. The critical point will therefore be reached at a much lower ver tilatory volume and the fall in arterial and alveolar carbon dioxide during voluntary hyperventilation will be much smaller. In these conditions the increased respiratory work at rest already leads to a statistically significant elevation of the basal metabolic rate. It is concluded that dyspnea can be objectively assessed with the study of the mechanism of ventilation.

J. HAAPANEN

The Efficiency of Ventilation During Voluntary Hyperpnea: Studies in Normal Subjects and in Dyspneic Patients with Either Chronic Pulmonary Emphysema or Obesity. J. FILLER*, A. P. FISHMAN, and A. COURNAND. *J. Clin. Invest.*, August, 1959, 38: 1339–1348. (*Bellevue Hosp., N.Y., N.Y.)

The efficiency value of the chest walls and diaphragms of 7 normal subjects was less than 7 per cent. The efficiency of these structures in 6 patients with emphysema was not greatly different at any particular work load, although the work produced a smaller exchange of air. By way of contrast, 5 obese patients had efficiency values which tended to be low. Since these low values were attributable to inordinately high energy costs, the data are best explained by presuming that this extra energy was required to move the adipose tissues overlying the chest and abdominal walls. The possibility that the muscles were abnormal cannot be excluded, although there is no evidence to support this view. Whatever the reason for the extra energy consumption, its expendi-

ture presumably contributed to the dyspnea these patients experienced on exercise.

E. Dunner

Essential Alveolar Hypoventilation with Cor Pulmonale (in German). W. Hadorn* and M. Scherrer. *Schweiz. med. Wchnschr.*, June 20, 1959, 89: 647–652. (*U. of Berne, Switzerland)

An imbecilic 38-year-old farm laborer developed cyanosis and signs of heart failure within a few weeks. Pulmonary function tests revealed a marked alveolar hypoventilation due to extremely irregular and shallow breathing. Mechanical causes, such as airway obstruction, lung tissue rigidity, impediment of the thoracic wall, or muscular weakness could be excluded. The ventilatory disturbance seemed to be related to an impaired sensitivity of the respiratory center to carbon dioxide. In the absence of other neurologic symptoms, the disease was called "essential" alveolar hypoventilation.

The pulmonary arterial pressure was elevated. It decreased essentially during artificial respiration, but increased markedly during oxygen breathing with carbon dioxide retention. A strict correlation existed between the pulmonary pressure gradient and the arterial carbon dioxide tension, but not between the arterial oxygen saturation and the pulmonary pressure gradient.

Treatment was successful with Engström's respirator. The patient is now working in the hospital without evidence of cyanosis.

J. Haapanen

Hemodynamic Variations Induced by Hypoxia in Patients with Pneumoconiosis (in Italian). F. Gobbato*, G. Sequi, G. Zagarese, and A. Berra. *Med. lavoro*, April, 1959, 50: 273–285. (*U. of Padua, Italy)

The hemodynamic modifications caused in silicotic patients by inhalation of a low oxygen mixture (10 per cent oxygen in nitrogen) for 16 to 22 minutes were studied in 14 cases to investigate the cardiovascular response to hypoxia, to find out if it was more intense in patients with pulmonary disease than in normal persons and, finally, to control the value of the Levy test (for hypoxia) in ascertaining a latent coronary artery disease.

The results were: there was a marked increase of cardiac output during hypoxia, due primarily to an increase of frequency rather than to an increase of systolic output; the hemodynamic response was more evident in persons with altered respiratory function; Levy's test was reliable not only in revealing a latent coronary artery disease, but also in visualizing electrocardiographic signs of an incipient defect of the right heart in persons with chronic pulmonary disease.

I. Archetti

Excretion of Electrolytes During Intermittent Positive-Pressure Respiration. J. Marshall*. *Brit. M. J.*, July 25, 1959, No. 5142: 85–87. (*Institute of Neurology, London, England)

The diurnal excretory tides of potassium, sodium, and chloride during periods of recumbency up to 2 years were unaltered in 5 patients. Periods of intermittent positive-pressure respiration of 7 days or more flattened first the excretory tide of potassium in 4 of 5 cases, and later those of sodium and chloride. Possible mechanisms altering the diurnal tides are discussed (Author's summary).

E. A. Riley

Treatment of Tetanus Neonatorum with Intermittent Positive-Pressure Respiration. P. M. Smythe* and A. Bull. *Brit. M.J.*, August 1, 1959, No. 5143: 107–113. (*U. of Capetown, and Groote Schuur Hosp., Capetown, South Africa)

A modification of the Radcliffe intermittent positive-pressure respirator is described which allows prolonged assisted respiration to neonates. Nine cases of tetanus neonatorum are described. Two children recovered completely. Two others survived for 7 weeks and died from causes other than tetanus. In the preceding 6 years prior to instituting this form of therapy, there were 40 cases of neonatal tetanus with only 1 survival. Additional treatment consisted of paraldehyde to control muscle spasm, tracheotomy, repeated aspirations, and muscle relaxants, such as mephenesin and tubocurarine chloride. Antimicrobials were used to prevent pulmonary infections.

E. A. Riley

Pulmonary Ventilation in Infants Under One Year of Age. T. K. Oliver, Jr.*, R. S. Shaw, and W. E. Wheeler. *A.M.A. J. Dis. Child.*, June, 1959, 97: 774–784. (*Ohio State U., Columbus)

A spirometer adapted for measurement of pulmonary ventilation in small infants is described. Forty-eight normal infants ranging in age from 3 days to 8.9 months have been studied and their values are given in detail. Linear correlation exists between age, weight, and body surface area; and tidal volume, minute ventilation, and respiratory rate. Observed values of minute ventilation in these infants when compared with body surface area correlate well with observations made in older children and adults. The possibility of utilizing this technique in the evaluation of pulmonary disease in infancy is suggested (Authors' summary).

M. J. Small

A Hazard in the Use of the Beaver Pulmonary Ventilator. E. H. Franks*. *Brit. M.J.*, July 25, 1959, No. 5142: 91–92. (*Royal Infirmary, Cardiff, Wales)

Two cases are described in which dangerous cyanosis occurred as a result of an obsolete type of diaphragm being fitted to the inspiratory-expiratory valve of a Beaver Ventilator (Author's summary).

E. A. Riley

Assessment of Respiratory Failure in Poliomyelitis. R. V. Walley*. *Brit. M.J.*, July 25, 1959, No. 5142: 78–82. (*Ham Green Hosp., Bristol, England)

The successful treatment of respiratory failure in poliomyelitis calls for accurate assessment of respiratory function. The methods used in the management of 62 cases of respiratory and bulbar paresis due to poliomyelitis are discussed. The application of these methods to the assessment of the respiratory function of these cases is described (Author's summary).

E. A. Riley

Simple Bedside Test of Respiratory Function. T. H. Snider*, J. P. Stevens, F. M. Wilner, and B. M. Lewis. *J. A. M. A.*, August 1, 1959, 170: 1631–1632. (*Wayne State U., Detroit, Mich.)

The ability of a patient to blow out a match at a distance of 15 cm. was found to be a useful clinical test if conditions were duly standardized. This test was performed in 126 patients and shown to be well correlated with the results of two other tests of respiratory function, namely, the test for one-second vital capacity, and maximal breathing capacity.

H. Abeles

A Respiratory Unit: The Toronto General Hospital Unit for the Treatment of Severe Respiratory Insufficiency. H. O. Barber*, R. A. Chambers, H. B. Fairley, and C. R. Woolf. *Canad. M.A.J.*, July 15, 1959, 81: 97–101. (*Toronto General Hosp., Canada)

In the spring of 1958, a special unit was established at the Toronto General Hospital to treat patients with severe respiratory insufficiency with intermittent positive pressure artificial respiration. In the first 5 months of the unit's operation, 21 patients were treated, and 2 died. The clinical conditions encountered were poliomyelitis, postoperative respiratory failure, stove-in chest, barbiturate overdosage, myasthenia gravis, emphysema, tetanus, and status epilepticus. During the 5 months prior to opening the unit, 17 patients with respiratory failure due to similar conditions were treated; 6 died. Neither of the 2 deaths occurring after the unit was established was due to respiratory failure. The team in charge of the unit includes a group of specially trained nurses, 4 physicians, a neurologist, a chest physician, an anesthetist, and an ear, nose, and throat surgeon.

E. A. Riley

Variability of Some Characteristics of Respiratory Mechanics in Normal Adults. L. Ehrner* and O. Nisell. *Acta med. scandinav.*, June 6, 1959, 164: 95–103. (*Serafimerlasarettet, Stockholm, Sweden)

Sixteen males and 15 females between 16 and 79 years of age with no evidence of past or present cardiopulmonary disease were studied by means of time-marked tidal volume esophageal pressure diagrams. The compliance ranged from 0.09 to 0.35 liter per cm. of water, the resistance from 1.5 to 3.6 cm. of water per liter per second, and the intrathoracic pressure variation during one breath from 3.6 to 11.9 cm. of water. The nonelastic work of breathing varied more than the compliance, resistance, or intrathoracic pressure variation, both among the subjects and during the studies. The compliance showed correlation with the body height, vital capacity, functional residual capacity, total lung capacity, and maximal breathing capacity. There was a slight negative correlation between resistance and weight. Age did not seem to influence the compliance or resistance values. The mean coefficients of variation for resistance and compliance from the curves during an examination and from repeated studies of the same subject varied between 12 and 18 per cent.

E. Dunner

MICROBIOLOGY AND IMMUNOLOGY

Fluorescence and Relative Acid-fastness of Tubercle Bacilli (in French). J. Degombier*. *Ann. Inst. Pasteur*, June, 1959, 96: 724–732. (*Pasteur Institute, Paris, France)

The Ziehl-Neelsen technique does not permit an absolute distinction between acid-fast and non-acid-fast bacilli. On a smear of authentic tubercle bacilli (H37Rv), one may see a majority of bacilli stained red, and a certain number of less intensely colored organisms, some of them a very pale pink. Fluorescence microscopy permits distinguishing bacilli not demonstrable by the Ziehl-Neelsen method. The importance lies in the fact that such feebly-stained bacilli are most probably young organisms. Studies in 198 sanatorium patients revealed 7 per cent more positive smears with fluorescence microscopy than with the Ziehl-Neelsen technique.

V. Leites

The Nicotinamidase Test for Differentiation of Human and Bovine Tubercle Bacilli (in German). R. Bönicke* and B. P. Lisboa. *Tuberkulosearzt*, June, 1959, 13: 377–384. (*Borstel-Institut, Borstel/Bad Oldesloe, Germany)

The technique of the nicotinamidase test is described. It is based on the ability of the bacilli to deamidize nicotinamide. The ammonia liberated by the enzyme is determined chemically with Russel's method; a bluish-green color indicates the presence of ammonia. The advantages of this method over Nessler's reaction are pointed out. The method was used to differentiate 25 strains of *M. tuberculosis* (10 human and 15 bovine) which were difficult to identify by the usual methods. Human bacilli exhibit a considerably higher deaminase activity than the bovine type.

J. Haapanen

Common Antigenic Components of Mycobacterial Extracts. J. Pefys*, R. Augustin, and A. B. Paterson. *Tubercle*, June, 1959, 40: 163–172. (*Brompton Hosp., London, England)

Protein and polysaccharide preparations from a variety of mycobacteria and human and rat lepromins were studied in agar-gel diffusion tests for the presence of antigenic components capable of reacting with a rabbit antiserum produced in response to the injection of killed *M. tuberculosis* H37Rv in Freund's adjuvant.

Three common precipitating antigens were found in the PPD's of *M. tuberculosis* (human, bovine, and avian strains), *M. johnei*, *M. phlei*, and human lepromin. Two of these common precipitating antigens were found in the culture filtrate and liquid paraffin extract polysaccharides. The rat lepromin contained two precipitating antigens in common with the PPD and polysaccharide preparations. Tests with proteolytic digests of the PPD preparations of human and bovine *M. tuberculosis*, *M. johnei*, and carbohydrate strains of the agar-gel precipitation lines suggest that polysaccharide components were responsible for the production of the precipitation lines.

M. J. Small

EXPERIMENTAL PATHOLOGY

Experimental Research on the Importance of Some Nonspecific Factors in the Pathogenesis of Diffused Pulmonary Fibrosis (in Italian). C. Pana*, G. Spina, M. Lucchesi, and P. Lo Pane. *Ann. Ist. Carlo Forlanini*, No. 3, 1958, 18: 321–330. (*U. of Siena, Italy)

In order to study the pathogenesis of pulmonary fibrosis, three groups of guinea pigs were treated either with inhalation of tobacco smoke for 10 minutes, or with a concentration of 1 per thousand of histamine by aerosol for 5 minutes, or multiple inoculations of horse serum.

Despite some differences found in the histologic picture of the animals belonging to the three groups (heavy hemorrhages in those treated with histamine and smoke, more evident mesenchymal response in the animals treated with serum), final morphologic results were similar: a condition of prefibrosis which never reached its complete evolution with the formation of a true fibrous tissue.

I. Archetti

Use of Monocyte Cultures for Controlling the Activity of Antituberculosis Drugs (in Italian). G. Daddi*, F. Basilico, and C. Grassi. *Gior. ital. tuberc.*, May–June, 1959, 13: 123–128. (*U. of Milan, Italy)

The *in vitro* culture of rabbit monocytes in Hanks' solution according to the Mackaness technique was employed to test the antimicrobial activity of streptomycin, isoniazid, cycloserine, and kanamycin against *M. tuberculosis*, strain H37Rv.

A complete inhibition of growth was obtained with 50 γ of streptomycin, 0.1 γ of isoniazid, 40 γ of cycloserine, and 30 γ of kanamycin, per ml. A bacteriostatic action, limited to 24 to 48 hours, was caused by 25 γ of streptomycin, 0.05 γ of isoniazid, 30 γ of cycloserine, and 20 γ of kanamycin, per ml. No toxic action against the cells could be demonstrated with the different antimicrobials.

I. Archetti

The Histopathology of *Mycobacterium Johnei* Infection in Small Laboratory Animals. H. P. Harding*. *J. Path. & Bact.*, July, 1959, 78: 157–169. (*U. of Liverpool, England)

Mice, rabbits, and hamsters were infected with fluid cultures of *Mycobacterium johnei*. Mice were inoculated intravenously and sacrificed from 6 to 614 days after infection. Rabbits were inoculated by oral, intraperitoneal, and intravenous routes. They survived 54 to 295 days. The hamsters were 2 days to 4 months old at the time of infection and were inoculated by the oral and intraperitoneal routes. After death the general character of the lesions was similar in all species, but there was a marked variation in organ susceptibility. In mice the commonest lesions were in the liver parenchyma; in rabbits and hamsters the portal tracts were more commonly involved. Pulmonary lesions were marked in rabbits; in hamsters and mice the changes were much less apparent.

H. J. Henderson

The Sero-immuneconglutinin in Silicosis (in Italian). B. Pernis*, G. Gambini, and M. Finulli. *Med. lavoro*, April, 1959, 50: 250–256. (*U. of Milan, Italy)

Serum specimens of 29 normal persons, 66 silicotic patients, and 7 patients with silicotuberculosis were tested, using a slight modification of Coombs' technique, for the presence of immuneconglutinin.

The values obtained in the normal persons agreed quite closely with those found by Marks and Coombs, and rarely reached a titer higher than 1:4. Among silicotic patients, 88 per cent had a titer equal to or higher than 1:8, and about half of the cases had titers from 1:32 to 1:128. An increase of the immuneconglutinin level was found in 6 of 7 patients with silicotuberculosis.

I. Archetti

Serum Proteins and Glycoproteins in Pulmonary Silicosis (in Italian). L. Buscarini* and F. Necrosini. *Med. lavoro*, May, 1959, 50: 368–378. (*U. of Pavia, Italy)

The serum protein and glycoprotein pattern was studied in 29 patients with pulmonary silicosis at the three different stages. According to the data obtained, silicosis always caused hyperproteinemia with an increase of α-2- and γ-globulins, and the former two were slightly more marked in patients in the third stage of the disease. There was a progressive increase in carbohydrates bound to total proteins and to α-2-globulins in the three stages; the increase of those bound to γ-globulins was constant. The mean value of the ratio of the α 2-globulin glycides to the α-2-globulins showed a clear and statistically significant increase in patients in the third stage.

The importance of the glycoprotein pattern in following the evolution of the disease is emphasized.

I. Archetti

Quartz-Agglutination Reaction in the Serum of Patients with Silicosis (in German). H. W. Schlipköter*, W. Thews, and R. Dolgner. *Deutsche med. Wchnschr.*, May 15, 1959, 84: 939–941. (*Medizinische Akademie, Düsseldorf, Germany)

The authors tested 350 serum specimens of patients with silicosis and of control subjects for the possible development of antibodies against quartz. Quartz dust was used as antigen, and the agglutination reaction was performed according to the method of Ishinishi. No correlation could be established between the results of the agglutination reaction and the development of silicosis. There was no evidence of quartz antibodies in patients with silicosis. In animal experiments it could be shown that there occurs only a small augmentation of agglutination after parenteral administration of quartz. These results could be significantly changed by the immunologic reactions of the bacteria present in the dust.

J. Haapanen

The Protein and Electrophoretic Patterns of Rabbits Vaccinated with Formol-Inactivated Bovine Tubercle Bacilli and Subsequently Treated with Isoniazid (in Italian). G. De Simoni* and M. Caracciolo. *Ann. Ist. Carlo Forlanini*, No. 4, 1958, 18: 443–453. (*Istituto C. Forlanini, Rome, Italy)

Two groups of rabbits were vaccinated during a 40-day period with Formol®-inactivated bovine tubercle bacilli; the animals of one group were kept as controls and the others were orally given a daily dose of 15 mg. of isoniazid for a period of 42 days, starting after the end of vaccination. Samples of blood were taken at the end of vaccination and 38 and 51 days thereafter for determination of values of proteinemia, albumins, globulins and γ-globulins.

In the treated rabbits an increase of proteinemia and albumins, a decrease of globulins with a consequent increase of the ratio of albumins to globulins and, finally, an increase of γ-globulins were found. From these results, compared with those obtained in the controls, it is concluded that treatment with isoniazid improved the protein pattern, which had been previously modified by the vaccination, and favored the increase of γ-globulins.

I. Archetti

Fate of Tubercle Bacilli in Healthy, Prevaccinated, and Preinfected Guinea Pigs (in Italian). G. Curci*. *Arch. tisiol.*, May, 1959, 14: 347–357. (*U. of Naples, Italy)

The distribution of subcutaneously injected BCG and the virulent H37Rv strain of *M. tuberculosis*, both labeled with P^{32}, in guinea pigs was studied in healthy, prevaccinated, and preinfected animals. They were killed after different intervals and specimens of various tissues were collected to test the radioactivity with Geiger-Mueller's apparatus.

The radioactivity was found in the healthy guinea pigs mostly at the point of inoculation and in the collateral lymph nodes, and much less in the peripheral lymph nodes and in the viscera. In the other two groups of animals, radioactivity was detected for the most part at the site of inoculation; a very small amount was diffused in the internal viscera. In the latter 2 groups a greater quantity of P^{32} was found in the urine than was found in the healthy group; this result was per-

haps due to a bacterial lysis caused by the immune condition of the guinea pigs.

I. Archetti

Contribution to the Comparative Pathology of Tuberculosis (in German). R. Ivens*. *Beitr. Klin. Tuberk.*, December, 1958, 119: 259–271. (*Institut f. Vergl. Path. Berlin-Friedrichsfelde, Wilhelmstr. 4, Germany)

Of 24 hedgehogs (Erinaceus europeus), 12 were infected with bovine tubercle bacilli during the summer. When they were sacrificed 3 months later, pulmonary tuberculosis was found in each of 9 animals (1 was permitted to hibernate, 2 died prematurely). The remainder were inoculated during their time of hibernation in November. Six of them died before spring, the other 6 were killed on resuming normal existence. None of them had tuberculosis. The sole surviving animal infected in the summer was also killed after hibernation, and produced the same tuberculous findings as those infected and sacrificed in the summer.

The hedgehog takes almost no nourishment during hibernation; its heart beat decreases from 150 to 18 per minute, it breathes once in every 4 minutes, and its body temperature sinks from 35°C. to 3°C.

Z. Virágh

The Use of Skin Ulcer Measurements to Estimate Immunity Against Tuberculosis in the Guinea Pig. C. N. Iland* and R. M. Barker. *Tubercle*, August, 1959, 40: 235–244. (*Ayrshire Central Hosp., Irvine, Scotland)

In experimental studies, immunity to tuberculosis is usually assayed by protection tests in guinea pigs or mice: the mean survival rate of nonimmune animals is compared with that of the vaccinated group. Such tests take considerable time and require large numbers of animals. A simpler method based on intradermal sensitivity reactions is reported in this paper.

Normal nonimmune guinea pigs, after intradermal challenge with living vole bacilli, develop ulcerated swellings which reach a maximum in about 4 weeks and then heal. Guinea pigs vaccinated with BCG and then challenged with vole bacilli by the intradermal route also develop ulcerated swellings, but they are smaller, appear more rapidly, and heal more quickly than in the nonimmune animal.

The speeds of development of hypersensitivity and immunity by the guinea pig are reflected in the progress of these lesions, and a comparison of their measurements in nonimmune and vaccinated animals can be used for estimating the level of immunity. The comparison is more accurate if the effect of hypersensitivity is excluded, and this was attempted by simultaneously challenging the animals in different sites with both killed and living vole bacilli. The measurements of the lesions produced by the "dead" challenge were subtracted from those of the "living" challenge, and the resulting values were used in the assay.

Using the technique described, the method appears sensitive and precise. Statistically significant results can be obtained with a small number of animals and in a short time. The method, therefore, has advantages over mortality rate determinations.

M. J. Small

The Time Required for the Establishment of Effective Immunity after Vaccination with BCG: An Experimental Study (in German). H. Spiess*. *Deutsche med. Wchnschr.*, May 22, 1959, 84: 982–984. (*U. of Göttingen, Germany)

Three groups of 20 guinea pigs each were infected with a moderately virulent strain of *M. tuberculosis* 9, 20, and 39 days, respectively, after injection of 0.1 mg. of BCG dry vaccine. As measured by the incidence of tuberculosis and the period of survival, demonstrable protection developed only after more than 3 weeks. Five to 6 weeks after BCG vaccination, an effective protection against superinfection was present. At this time the tuberculin test was always positive. These data support the clinical practice of avoiding exposure to tuberculosis for at least 6 weeks after BCG vaccination.

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Canada	250
Foreign and Miscellaneous	3,227

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	No.	%
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Starred pulmonary specialist	84	19.27
Pulmonary specialist	24	5.50
Starred internist	85	19.49
Starred surgeon	31	7.10
Surgeon	3	.68
Starred thoracic surgeon	27	6.19
Starred radiologist	7	1.60
Starred public-health specialist	5	1.14
Starred pathologist	3	.68
Starred pediatrician	3	.68
Starred otologist	2	.45
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35–39	55	12.61
40–44	58	13.30
45–49	50	11.46
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100–200,000	32	7.33
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1	1	1	0	1	2	2	50–100,000
2	0	1	1	0	0	2	100–200,000
3	6	0	0	0	1	4	200–500,000
4	3	2	0	0	1	0	500–1,000,000
2	4	1	1	2	0	6	Over 1,000,000
18 4.12%	20 4.58%	15 3.44%	4 .91%	6 1.37%	8 1.82%	15 3.44%	86 19.72%

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